Extraction of Tamoxifen and Metabolites from Urine and Plasma using Supported Liquid Extraction (SLE) prior to LC-MS/MS Analysis

Lee Williams, Rhys Jones, Helen Lodder, Geoff Davies, Steve Jordan, Richard Calverley, Claire Desbrow & Gary Dowthwaite
Biotage GB Limited, Distribution Way, Dyffryn Business Park, Ystrad Mynach, Hengoed, CF82 7TS, UK.

Introduction

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 various human biological fluids.

Experimental Procedure

Reagents

Formic acid, ammonium hydroxide and tamoxifen were purchased from Sigma Chemical Co. (Poole, UK). 4-hydroxytamoxifen, N-desmethyltamoxifen and 4-hydroxy-N-desmethyltamoxifen (endoxifen) were purchased from Toronto Research Chemicals (North York, ON, Canada). Human plasma was obtained through the Welsh Blood Service (Pontyclun, UK). Urine was obtained from a healthy human volunteer. All solvents were HPLC grade from Fisher Scientific (Loughborough, UK).

Sample Preparation

Supported Liquid Extraction Procedure

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

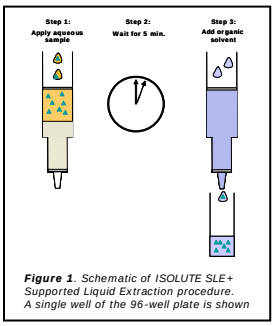

***Figure 1**. Schematic of ISOLUTE SLE+ Supported Liquid Extraction procedure. A single well of the 96-well plate is shown*

Sample pre-treatment: Plasma or urine (100 µL) was diluted (1:1 (v/v)) with either 1% formic acid aq, 0.1% formic acid aq, H_2O or 0.5M NH_4OH. Spike concentrations were 200 ng/mL of plasma.

Sample Application: The pre-treated plasma/urine (200 µL) was loaded onto the plate, a pulse of vacuum applied to initiate flow and the samples left to absorb for 5 minutes.

Analyte Elution: Addition of 1 mL of various water immiscible extraction solvents. The extraction solvents evaluated were Hexane, MTBE, EtOAc, DCM.

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 Conditons

Instrument: Waters Acquity UPLC (Waters Assoc., Milford, MA, USA).
Column: Acquity UPLC BEH C18 column (1.7µ, 100 x 2.1 mm id) (Waters Assoc., Milford, MA, USA).
Mobile Phase: 0.1% formic acid aq and 0.1% formic acid/MeOH at a flow rate of 0.5 mL/min.
Gradient: Isocratic 10%, 0.1% (v/v) formic acid aq and 90% 0.1% formic acid/MeOH.
Injection Volume: 5 µL.
Column Temperature: 35 ˚C.

Mass Spectrometry

Instrument: Quattro Premier XE triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive ions were acquired in the multiple reaction monitoring (MRM) mode.
Desolvation Temperature: 450 °C
Ion Source Temperature: 150 °C
Collision Gas Pressure: 3.56 x 10^{-3} mbar

Scan Function	Analyte	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. Quattro Premier XE mass spectrometer parameters

Results

Figures 1-4. demonstrate recoveries for the extraction of tamoxifen and metabolites from plasma. Recoveries > 70% were observed using a number of pre-treatment/extraction solvent protocols.

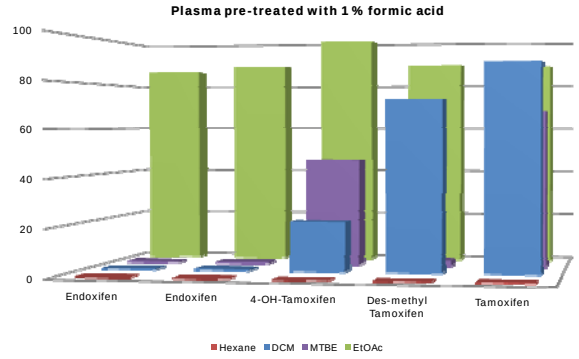

Figure 1. *Tamoxifen recovery profile from plasma using 1% formic acid pre-treatment.*

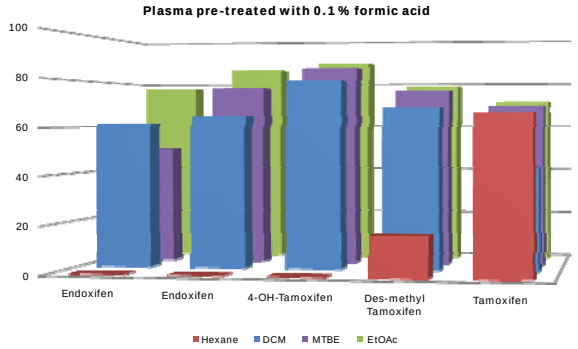

Figure 2. *Tamoxifen recovery profile from plasma using 0.1% formic acid pre-treatment.*

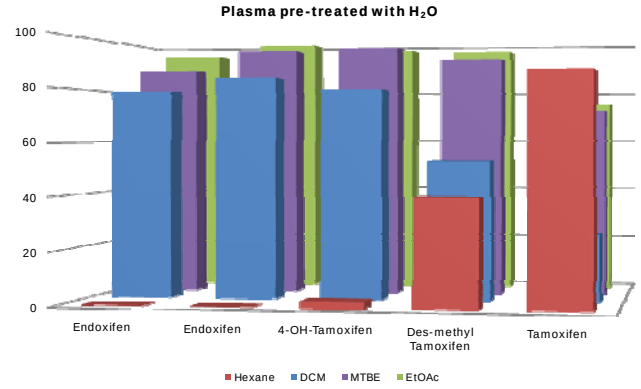

Figure 3. *Tamoxifen recovery profile from plasma using H2O pre-treatment.*

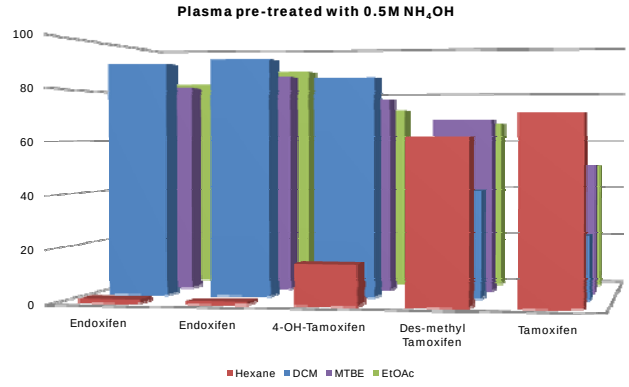

Figure 4.*Tamoxifen recovery profile from plasma using 0.5M NH_4OH pre-treatment.*

Figures 5-8. demonstrate recoveries for the extraction of tamoxifen and metabolites from urine. Once again a variety of extraction protocols resulted in recoveries > 70% with corresponding RSDs < 10%.

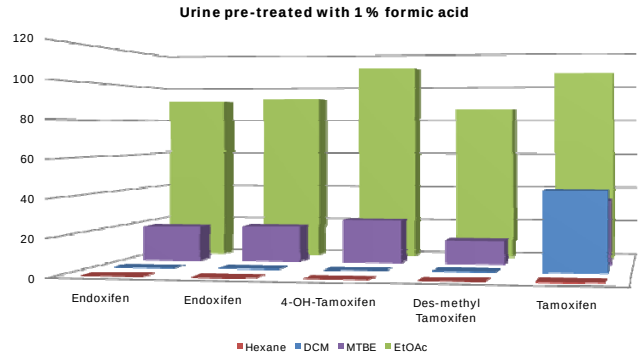

Figure 5. *Tamoxifen recovery profile from urine using 1% formic acid pre-treatment*

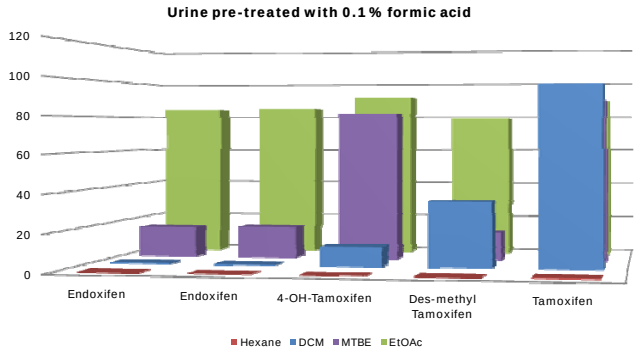

Figure 6. *Tamoxifen recovery profile from urine using 0.1% formic acid pre-treatment.*

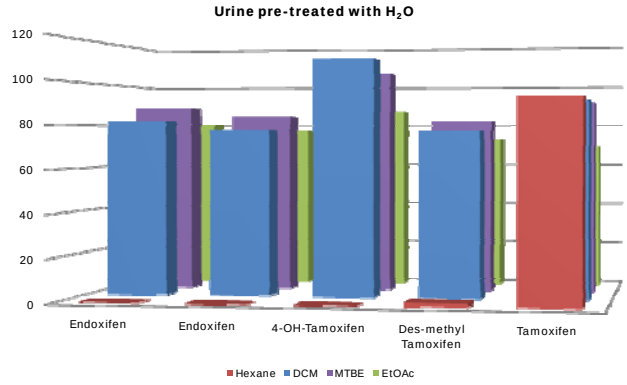

Figure 7. *Tamoxifen recovery profile from urine using H2O pre-treatment.*

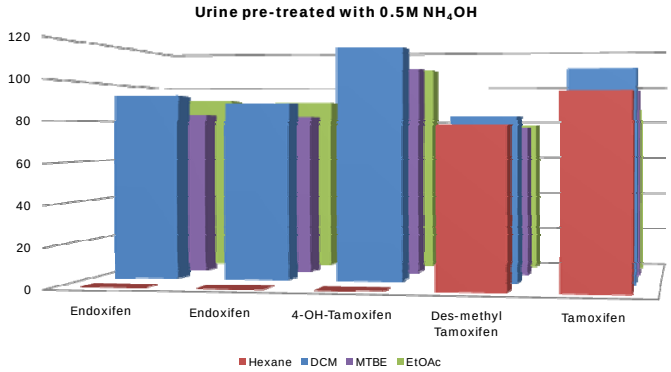

Figure 8. *Tamoxifen recovery profile from urine using 0.5M NH_4OH pre-treatment.*

Conclusions

1. High reproducible recoveries were observed using various extraction combinations.
2. Hexane showed high recoveries of the less polar tamoxifen and desmethyltamoxifen but very low extraction efficiencies for the more polar metabolites.
3. Higher extraction efficiencies were observed for tamoxifen and desmethyltamoxifen from urine, suggesting that protein binding may occur in plasma.
4. EtOAc alone demonstrated acceptable extraction efficiencies from urine using 1% formic acid pH pre-treatment however, at higher loading pHs multiple solvents delivered recoveries > 80%.
5. Plasma extracts exhibited higher efficiencies using either H_2O or 0.5M NH_4OH pre-treatment.
6. This poster shows the applicability of SLE+ to extract tamoxifen and metabolites from plasma and urine.
7. RSDs were below 10%, demonstrating reproducible extraction and recoveries.

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United States and Canada
Tel: +1 704 654 4900
Toll-Free: +1 800 446 4752
ordermailbox@biotage.com

United Kingdom, EIRE
Biotage
Tel: +44 1992 501535
order@eu.biotage.com

Sweden
Biotage
Tel: +46 18 56 59 00
order@eu.biotage.com

Japan
Biotage
Tel: +81 422 281233
order@biotage.co.jp