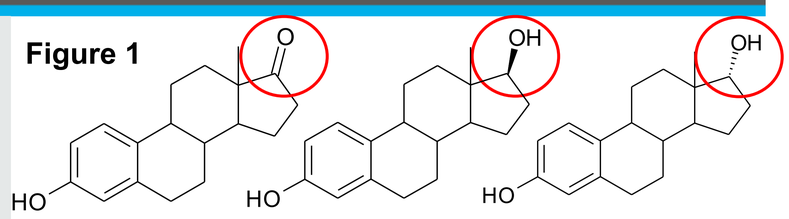
Derivatisation of estrogens enhances specificity and sensitivity of analysis of human plasma by liquid chromatography tandem mass spectrometry

Abdullah MM Faqehi, Diego F Cobice, Gregorio Naredo-Gonzalez, Tracy CS Mak, Brian R Walker, Natalie ZM Homer, Ruth Andrew
Endocrinology, University/British Heart Foundation Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh

Introduction

- Analysis of endogenous estrogens is a very difficult task due to their extremely low concentrations (<50 pg/mL in men and 30-50 pg/mL in postmenopausal women)
- Immunoassays have been widely use to quantify estrogens but suffer from interference due to similarity in chemical structure (Figure 1)

Figure 1



Estrone (E1) **Estradiol (E2)** **17α-Estradiol**

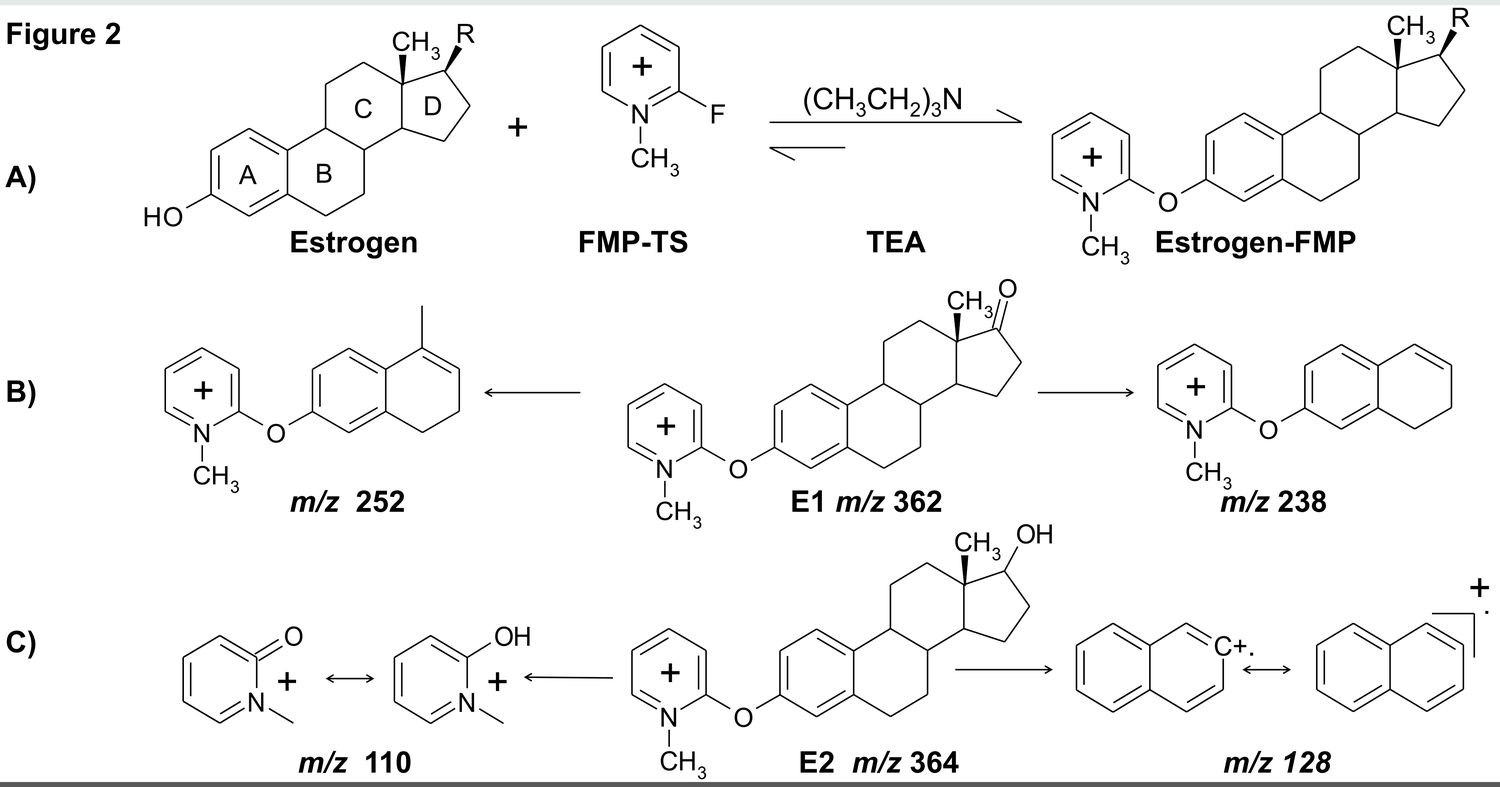
Analysis of estrogens

- Liquid chromatography-mass spectrometry (LC-MS) provides an alternative analytical method with derivatisation to boost sensitivity
- Disadvantage for tandem MS is that often the product ion of derivatised species is formed from the derivatising group and is not specific for the analyte by mass
- A derivative generating a specific product ion is desirable as it allows greater distinction from background isotope interference, particularly in conjunction with rapid chromatography
- **Aims:**
- To establish a derivative suitable for analysis of estrogens in human plasma, which yields precursor and product transitions specific to estradiol and estrone
- To develop a validated method using ion-exchange solid-phase extraction in order to detect low levels of estrogens in human biological fluids by tandem MS

Derivatisation and MS/MS fragmentation

- Formation of methylpyridinium ether derivative of phenolic estrogens was achieved and optimised using 2-fluoro-1-methylpyridiniump-toluenesulfonate (FMP-TS) in the presence of triethylamine (TEA) (Figure 2A)
- Derivatives for estrogens were tuned on a triple quadrupole mass spectrometer in MS/MS using ESI mode (Figure 2B,C)

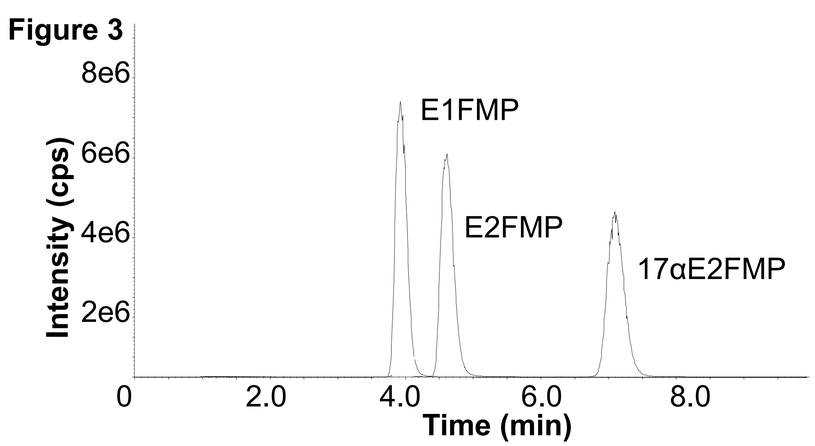
Figure 2



Chromatographic separation

- Baseline resolution of all analytes were achieved (Figure 3)
- Limit of quantitation (LOQ) for estrogens derivatives were determined and and compared favourably to the biological levels (Table 1)

Figure 3



Analyte	LOQ (pg)	Male		Female	
		pg/mL	pmol/L	pg/mL	pmol/L
E_1	5	30-50	150-250	30-250	150-850
E_2	5	25-50	100-250	100-400	500-1000

Table 1

LC method

- Acquity UPLC BEH C18 (2.1x 50mm, 1.7um) at 25°C
- Isocratic: (65:35) (water: methanol) +0.1% formic acid
- Flow rate 0.4 mL/min for 10 min

Method validation

- Linear standard curve of estrogen derivatives were generated in the range 5-1000 pg/mL (Figure 4 e.g. estrone calibration curve)
- The values of intra- and inter- assay precision and accuracy were acceptable (<20% RSD for precision and ±20% accuracy) (Table 2)
- Stability following storage in the auto-sampler (10°C) and freezer (-20 and -80°C) were assessed by injection after 24 and 48 hours and 28 days (Table 3)

Figure 4

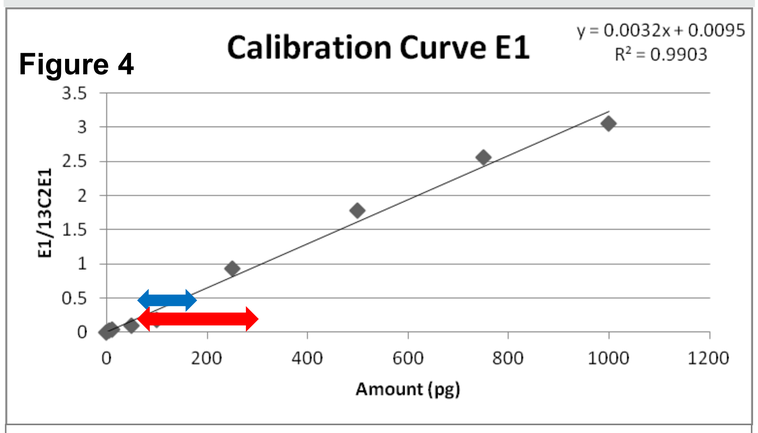


Table 2

Precision (%RSD)		
Analyte	E_1	E_2
Intra-assay	13	14
Inter-assay	15	16
Accuracy (%RME)		
Intra-assay	18	17
Inter-assay	19	19

Table 3

Degradation(%)			
Analyte	Time	E_1	E_2
10°C	24h	5	6
-20°C	24h	2.2	3.1
	48h	36	42
-80°C	24h	1.8	1.4
	48h	1.9	1.6
	28 d	6	8

Extraction

- The optimised method using ion-exchange solid-phase extraction extraction (Figure 5) was achieved with high recoveries (Mean ±RSD; E1 (95±5%) and E2 (92±3%))

Figure 5

Condition: 2mL methanol, 2mL water

Loading: 2mL sample

Washing: 2mL 5% methanol/water

Elution: 2mL methanol

Conclusion

- Derivatisation to form FMP ethers of phenolic estrogens, and analysis by LC-MS/MS, is suitable for quantitative analysis of these steroids in low abundance in biological fluids which improved specificity over previously reported derivatisation approaches
- Use of this approach allows robust measurement across typical physiological ranges found in men and premenopausal women
- Care should be taken to store derivatised samples at -80°C for periods greater than 24 h
- The derivatisation reaction may be applicable to other naturally occurring phenolic steroids