A novel uPLC-MS/MS method to quantify oestrogens and their sulphates optimised using MUSCLE software

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Introduction

Oestrogens are implicated in many diseases and drive cell proliferation in breast, ovarian and endometrial cancer. Sulphated oestrogens are inactive and represent a circulating reservoir for active oestrogens. LC-MS/MS methods are the gold-standard for steroid measurements(1). Thus, MS methods that can accurately quantify oestrogens and their sulphates are vital for understanding oestrogen-related disease.

Methods

The method was developed from Owen et al 2013(2). Samples with representative internal standards were extracted using Isolute solid phase extraction columns and analysed by Waters Xevo LC-MS/MS in negative ion mode with 0.3mM ammonium fluoride (aqueous phase). Optimal separation of oestrone (E_1), oestradiol (E_2), oestrone-sulphate (E_1S) and oestradiol-sulphate (E_2S) was performed using MUSCLE software (Multi-objective Unbiased optimisation of Spectrometry via Closed Loop Experimentation), (Figure 1 and Table 1). MUSCLE software is a recent development by Bradbury et al.(3) (**www.muscleproject.org**) and involves automated optimisation of targeted LC-MS/MS analyses.

To test the method, colorectal cancer (CRC) cell lines HCT116, HT-29 and Colo205 were treated for 1 hour with E_1, E_2 and E_1S (20, 100, 200, 400 and 800 nmol/l). Oestrogen metabolism was then compared across cell lines.

Figure 1.

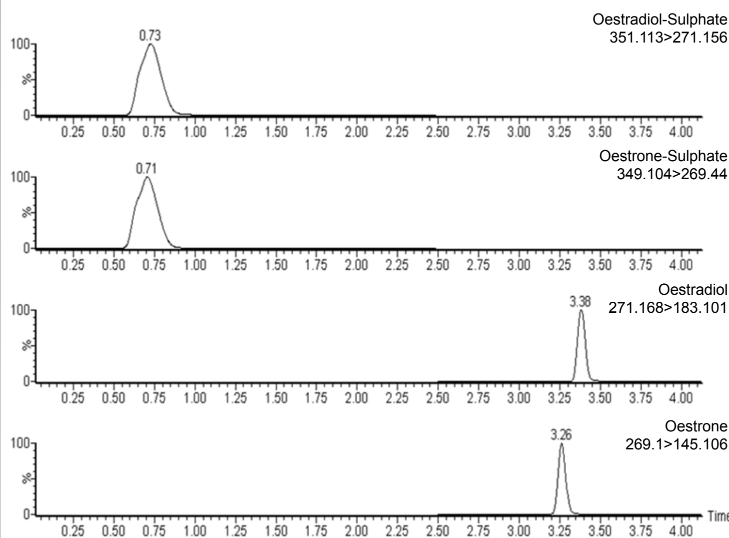

Figure1. Chromatogram from the MUSCLE optimised method for oestrone (E_1), oestradiol (E_2), oestrone-sulphate (E_1S) and oestradiol-sulphate (E_2S).

Table 1.

Time (min)	Flow	Water (%)	Methanol(%)	Gradient
Initial	0.45	70	30	
2.20	0.45	50	50	6
5.00	0.45	0	100	6

Table 1. Table showing the changes in methanol (%) over time and gradient

Results

The MUSCLE optimised method analysed E_1, E_2, E_1S and E_2S over the linear range 0.5-500 ng/ml in less than 5 minutes (Figure 2). Average recovery for all oestrogens was approximately 100%. Variability of repeated extractions at high and low concentrations (CV%) are shown in Table 2.

Table 2.

	E_1	E_2	E_1S	E_2S
Low Concentration (CV%)	5	15	9	14
High Concentration (CV%)	5	4	9	8

Table 2. Low concentration (CV%) at 18nmol/l for E_1 and E_2 and 14nmol/l for E_1S and E_2S and high concentration (CV%) at 554nmo /l for E_1 and E_2 and 427nmol/l for E_1S and E_2S.

Figure 2.

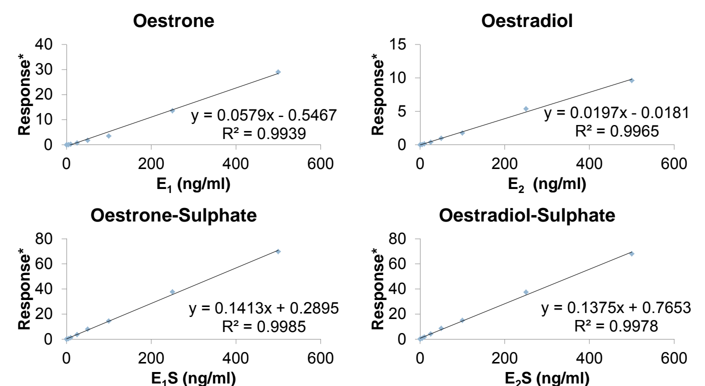

Figure 2. Calibration curves across the linear range 0.5-500ng/ml for oestrone, oestradiol, oestrone-sulphate and oestradiol-sulphate.
*Response is peak area divided by internal standard area

Oestrogen metabolism was detected in the three CRC cell lines after treatment with E_1, E_2 and E_1S.

Figure 3.

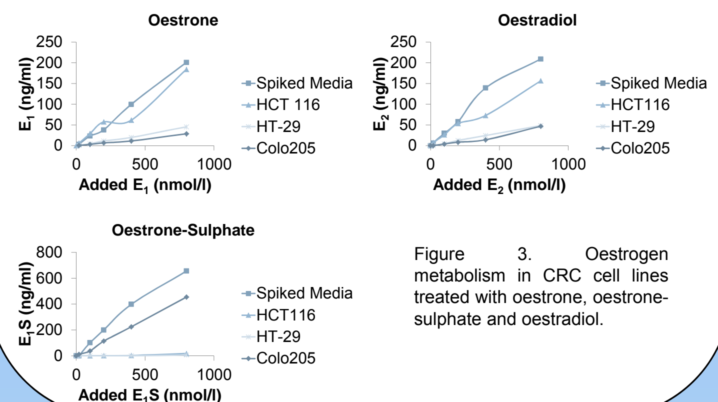

Figure 3. Oestrogen metabolism in CRC cell lines treated with oestrone, oestrone-sulphate and oestradiol.

Conclusions

- MUSCLE software enabled the rapid development of this novel highly specific, high throughput method that accurately quantifies E_1, E_2 and their sulphates together in less than 5 minutes.
- Validated and tested on cell cultures
- Could also be applied to tissue, serum and urine based research.
- In the future other oestrogen metabolites, such as 16α-hydroxyestrone and 2-hydroxyestrone, could be added to this method.
- This method will significantly benefit future oestrogen-related research

References

1. Handelsman DJ and Wartofsky L. JCEM. 2013; 98(10): 3971-3973
2. Owen LJ, Frederick W, Keevil BG. ANN Clin Biochem. 2013
3. Bradbury J, Genta-Jouve G, Dunn WB, O'Hagan S, Goodacre R, Knowles JD and Viant MR. 9th Annual Conference of the Metabolomics Society, Glasgow 2013.

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