

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.

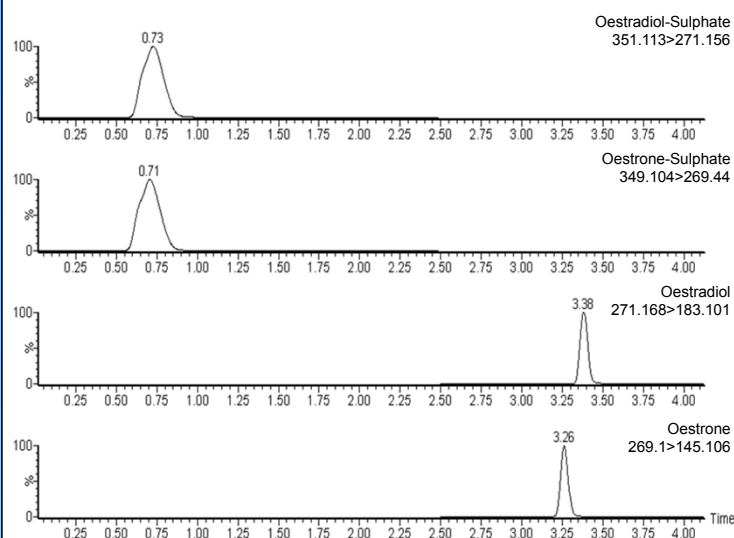


Figure1. Chromatogram from the MUSCLE optimised method for oestrone (E_1), oestradiol (E_2), oestrone-sulphate (E_1S) and oestradiol-sulphate (E_2S).

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 554nmol/l for E_1 and E_2 and 427nmol/l for E_1S and E_2S .

Figure 2.

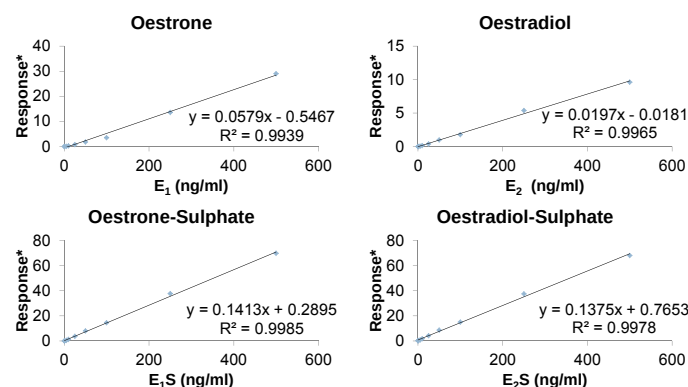


Figure 2. Calibration curves across the linear range 0.5-500ng/ml for oestrone, oestradiol, oestrone-sulphate and oestradiol-sulphate.

*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.

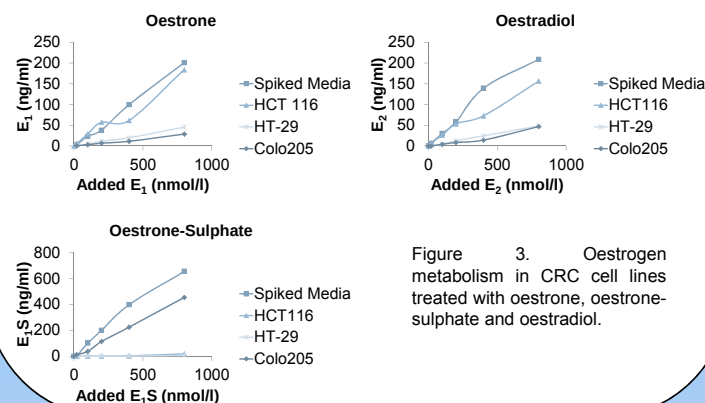


Figure 3. Oestrogen metabolism in CRC cell lines treated with oestrone, oestrone-sulphate and oestradiol.

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 16 α -hydroxyestrone and 2-hydroxyestrone, could be added to this method.
- This method will significantly benefit future oestrogen-related research

References

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- Owen LJ, Frederick W, Keevil BG. ANN Clin Biochem. 2013
- 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.

Acknowledgements

