Poster

[P27-8] P27-8: Assay and monitoring

Chair: Yoshihiko Hirotani, Japan

Wed. Sep 27, 2017 12:30 PM - 1:30 PM Annex Hall (1F)

(Wed. Sep 27, 2017 12:30 PM - 1:30 PM Annex Hall)

[P27-8-3] High-sensitivity determination of underivatized estradiol in human serum by a validated LC-MS/MS method

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Keywords: high-sensitivity, underivatized, estradiol, LC-MS/MS

Background

Accurate measurement of estradiol (E_2) at low concentrations is required in postmenopausal women, men, pediatric patients, and to assess the efficacy of antierstrogen therapies. This study aims to develop and validate a high-sensitive analysis of underivatized E_2 in low volume of human serum.

Methods

E_2 and d_3-E_2 (internal standard) were extracted from 200 L of human serum with 1-chlorobutane. Chromatographic separation was performed on C18 analytical column under gradient elution with mobile phases consisting of methanol (phase A) and 0.2 mM ammonium fluoride (phase B). Negative electrospray ionization and multiple reaction monitoring were used to follow the predominant transitions: collision energy (CE) -64, m/z 271→143 (qualifier for E_2); CE-50, m/z 271→145 (quantifier for E_2), CE -50, m/z 274→145 for d_3-E_2. Raw data of mass chromatograms were collected and processed by specialized software, and weighted (1/X) linear regression was performed to determine the concentration of E_2. Validation strategy was adhered to current industrial and clinical guidance.

Results

Selectivity was assessed with 15 individual native matrices of human serum applying the technique of standard additions: 5 from postmenopausal women, 5 from men, and 5 from premenopausal women, at each calibration point, in the range 2 –1000 ng/L, and associated with normalized matrix effect averaging 90–111% (percent matrix bias: -10 ÷11%), and imprecision within 15%. Inaccuracy ranged from -10.0 to 6.9 % within runs and from -14.7 to 11.3 % between runs. Imprecision was up to 12.7% within-runs, and up to 14.8% between-runs. Linearity was assured in the range 1.0 ÷1000 ng/L, R^2>0.996. Freeze-thaw stability was determined for three cycles each lasting 24 h, post-preparative stability was documented for 24 h at 4°C, short-term stability at room temperature was proven for 6 h at daylight and 4 h in the dark; stock solution stability and long term stability in serum were documented for 96 days at -20°C. With run time of 6 min, a throughput of over 100 samples per working day was achieved.

Conclusions

The method was validated according to current industrial and clinical requirements and allows the accurate and precise determination of E_2 in human serum.