CHEMOMETRIC APPROACH TO THE OPTIMIZATION OF LC-MS/MS SYSTEM PARAMETERS FOR ESTROGENIC HORMONE ANALYSIS

Can Aftafa A, Binnaz ŞahintürkB, Füsun Okçu Pelit A, İlgi Kapdan B, F. Nil Ertaş A*

A Ege University, Faculty of Science, Department of Chemistry, 35100, İzmir, Turkey
B Dokuz Eylül University, Dep. of Environmental Engineering, Izmir, Turkey
*E-mail: niler1919@hotmail.com

Natural steroid estrogenic hormones, estrone (E1), 17β-estradiol (E2), estriol (E3) secreted by humans and animals and the synthetic ethynylestradiol (EE2) used by women were identified as having the highest endocrine disrupting potential [1]. There is an urgent need to develop well-established analytical methods in water samples at at ng/L or even pg L⁻¹ levels [2]. LC methods coupled with MS detectors have recently been preferred in hormone analyses [3].

Literature survey has revealed that the studies for optimization procedures in LC-MS systems aim to improve the lowest limit of detection (LOD) values. They were usually achieved studying each factor separately in a traditional trial and error method. In addition, sophisticated instruments provide an ease of self-optimization in many applications. These methods require handling a number of parameters. However, a statistical method based on the use of an experimental design will give an insight about any interaction between the factors and the number of experiments required can also be reduced.

This paper describes the use of experimental design in LC-MS-MS method for selective and sensitive determination of natural estrogens namely; estradiol (E2), estrone (E1), estriol (E3) and synthetic estrogen as ethynyl estradiol (EE2) for the first time. Preliminary studies were dedicated to the statistical experimental design for optimization of mobile phase composition, LC elution and LC-MS/MS operation conditions by using Box Behnken response surface methods. The volume ratio of NH₄OH in the mobile phase was selected as 3%. The predicted optimal LC elution conditions were estimated as %ACN_standard: 28, %ACN_mobile: 44 and flow rate was 137. The optimal operation conditions of MS/MS detector system were adjusted as follows: Sheath gas pressure: 33 Arb, ion sweep gas pressure: 0.4 Arb, aux gas pressure: 17 Arb, capillary temperature: 254°C, vaporizer temperature: 352°C, collision gas pressure: 1.9 mTorr, spray voltage: 2740 V. The chromatograms recorded under optimized conditions have revealed that peak symmetry, resolution factor and peak area of the hormones were substantially improved with a signal gain about 20-25 times with respect to the instrumental self-optimized conditions. The proposed experimental conditions were validated using deionized pure water samples and linear calibration graphs were obtained for all hormones whose regression coefficients were larger than 0.98. RSD values were found less than 10% for three levels of concentration. LOD levels were calculated as; 1.7, 49.1, 3.3 and 23.2 ng L⁻¹ for E1, E2, E3 and EE2, respectively. The method developed was applied for water samples by using QuEChErS sample preparation technique. It provides that estrogenic hormone can be measured by this means at ppt level.

KEYWORDS: Estrogenic hormones, LC-MS/MS, Optimization, Box Behnken Design

REFERENCES: