LC/MS/MS METHOD DEVELOPMENT FOR IDENTIFICATION AND QUANTIFICATION OF 17B-ESTRADIOL IN WASTE WATER

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Abstract. *Many chemicals which are released into the environment and have the potential to disrupt the function of endocrine systems are called endocrine disrupting chemicals. A category included in these EDCs is the estrogens. A significant representative of this category is 17β-estradiol which is excreted by both male and female. Its presence in waste water is very dangerous because, following the path of waste water; it goes to rivers causing significant damage to fish environment. Due to its importance, a method was developed for screening and confirming 17β-estradiol residue in waste water samples. Method development consisted in preparing the standard solutions, optimizing the compound and source dependent parameters, choosing the mobile phase and the appropriate HPLC column followed by processing a calibration curve and finally injecting a real sample. The waste water sample was extracted according to some bibliographic studies, then injected on a Phenomenex Luna C18 column and detected through a LC/MS/MS system in an ESI (electrospray ionization) negative mode. The results were considered to be satisfying.*

Keywords: 17β-estradiol, waste water, solid phase extraction (SPE), electrospray ionization.

INTRODUCTION

Endocrine-disrupting chemicals (EDCs) are broadly defined as chemicals which may interfere with the function of the endocrine system in wildlife and humans [1-5]. Endocrine disruption causes fish feminization at approximately the ng L−1 level [6, 7] is considered to be linked to human cancers and may also affect human fertility [1–5]. A variety of commonly used chemicals having endocrine-disrupting properties are the sex hormones (estrogens, progestogens, and androgens) which carry the most estrogenic potency [8]. One of the estrogens that have endocrine-disrupting properties is 17β-estradiol (figure 1) which is a sex hormone naturally excreted not only by women and female animals, but also by men [8]. It has the chemical formula $C_{18}H_{24}O_2$, the molecular weight 272.38 g/mol and a very low solubility in water [9].

Due to the aspects presented earlier, the study of 17β-estradiol and its impact on human health and aquatic environment became very important, justifying the purpose of this study which is to develop a sensitive, reproducible and economic LC/MS/MS method allowing quantifying the presence of 17β-estradiol in waters.

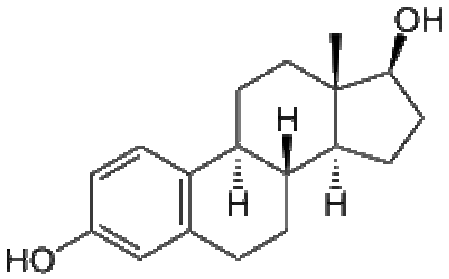

Fig.1 Chemical structure of 17β-estradiol

Experimental

Reagents and standards

The method developed in this study was accomplished using 17β-estradiol analytical standard (≥98.0%), Methanol LC-MS Optigrade (≥99.8%) and Acetonitrile LC-MS Optigrade (≥99.8%) acquired from LGC Standards. NaOH analytical reagent (99%) was acquired from Fluka. The ultra-pure water was obtained with a Milli-Q water purification system from Millipore.

The sample extraction was performed using Strata C18-E (55um, 500 mg, 12mL) solid phase extraction (SPE) cartridges and Phenex-RC 4mm Syringe Filters 0.45 µm obtained from Phenomenex.

Standard Solutions Preparation

Stock Solutions (~ 1mg/mL) was prepared dissolving 5 mg of 17β-estradiol in 5 mL of MeOH using a vortex mixer. In order to optimize the compound dependent parameters (infusion) of the source a solution 10 µL of stock solution were diluted in 100 mL MeOH. The source dependent parameters (FIA-flow injection analysis) were optimizing using a solution made from 10 µL of stock solution diluted in A/B 10:90 v/v, where A represents water and B represents ACN.

Sample extraction

The sample was prepared before extraction by adjusting its pH to 7 with NaOH and then filtered using 0.45 µm syringe filters. Then the cartridge was conditioned with 5mL methanol, followed by 5mL of Milli-Q water. Sample volume of 250 mL was passed through the cartridge at a flow rate of 10–15mL/min. The analytes retained were eluted using 5mL of MeOH (with 5% ACN). Extracts were evaporate to dry and final extracts were redissolved with 1mL of MeOH:H2O (80:20). After being filtered through 0.45 µm syringe filters, 50µL of this solution was injected into the chromatographic system [10].

Liquid Chromatography conditions

An HPLC system Agilent 1200 Series, consisting in an autosampler Agilent G1316A, column oven G1316A and a binary pump G1312A, was used. The liquid chromatographic column was a Phenomenex Luna C18 100A, 3µm, 50×2.0 mm. The cromatography was performed by isocratic reverse phase separation with a 5µL injection volume, using a mobile phase of A/B 10:90 v/v at a flow rate of 0.2 mL/min. The column oven was set to 20ºC.

Mass Spectrometry detection

The mass spectrometry detection was carried out with an API 3200 QTRAP LC/MS/MS system using a TurboV in an ESI (electrospray ionization) mode. Multiple reaction monitoring (MRM) transitions were followed to quantify the deprotonated precursor molecular ions $[M+H]^+$ and the related product ion.

Results and Discussion

For LC/MS/MS method development several steps must be fulfill. These steps are: choosing the TurboV source ionization (ESI or APCI); choosing the ionization mode (positive or negative); Q1 and Q3 scanning; establishing the compound dependent parameters by infusion; establishing the mobile phase; establishing the source dependent parameters by performing FIA; selecting the column which determinate the flow and the analysis time; performing a calibration curve and injecting real samples.

In order to choose the appropriate method to determine 17β-estradiol from real samples, the study started with the selection of TurboV source ionization. APCI and ESI were evaluated both in positive and negative modes and ESI negative ion mode was found to be the most effective for this compound.

Knowing the molecular mass of 17β-estradiol (272.38), a Q1 negative scanning using the infusion solution shows its presence at 271.1 m/z ratio (figure 2). The compound obtained in Q1 was fragmented in Q2 and the product ion was obtained in Q3 at 144.8 (figure 3).

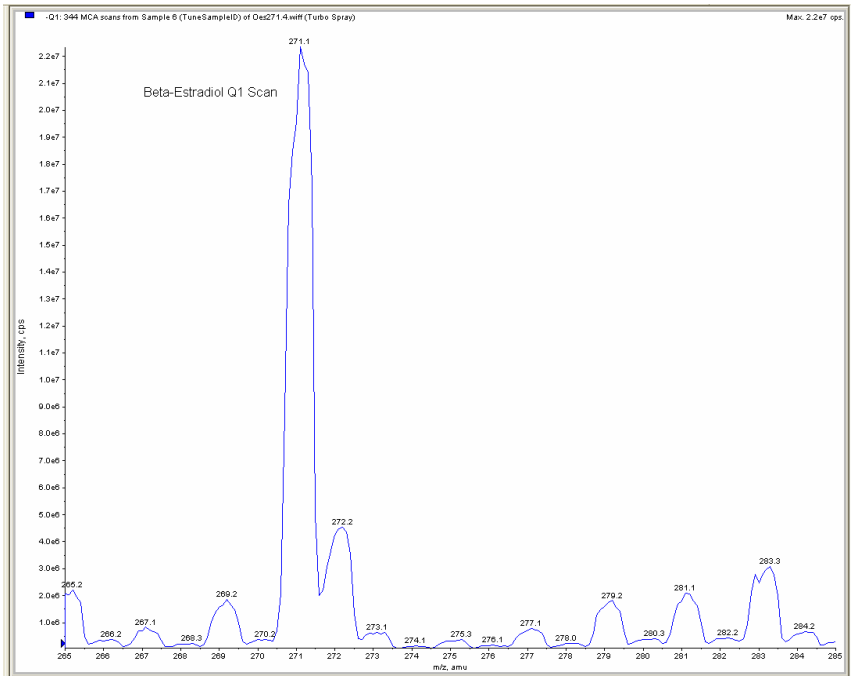

Fig. 2. Chromatogram obtained for 100 ng/mL 17β-estradiol in infusion solution by Q1 negative scanning mode.

The next step was to optimize the compound dependent parameters of the source using the infusion solution with an injection flow of 5µL. The following parameters were obtained:

DP (Declustering potential): -60V;

EP (Entrance potential): -9.5V;

CEP (Collision cell entrance potential): -16.0V;

CXP (Collision cell exit potential): -0.5V.

The precision of the obtained parameters was check by using ramp parameter settings, individually, for every one of them. The results proved their accuracy.

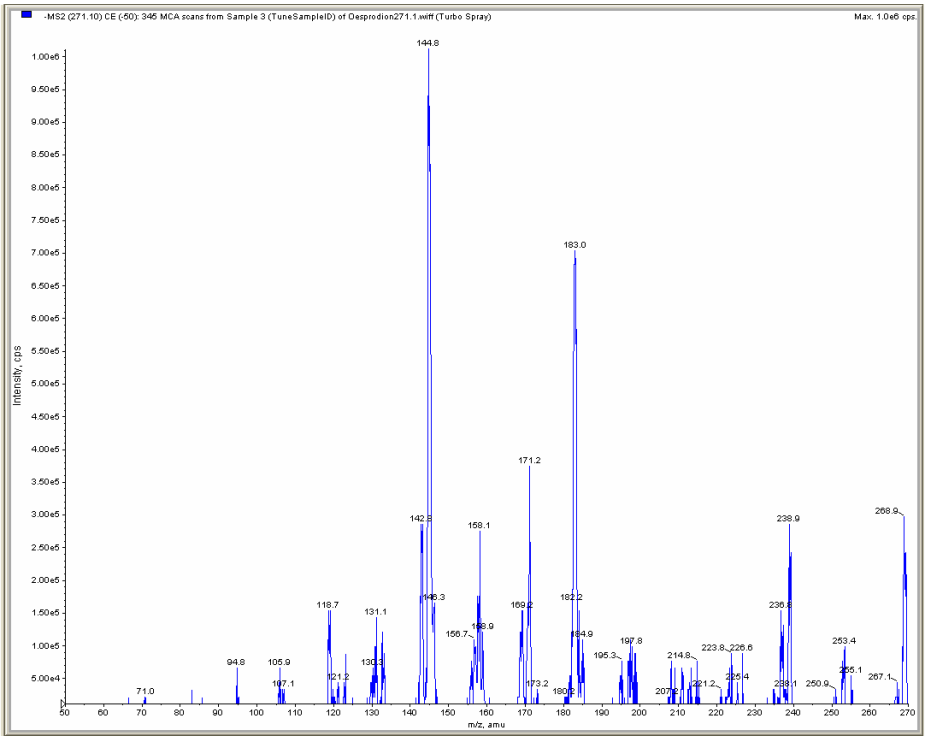

Fig. 3. Chromatogram obtained for 100 ng/mL 17β-estradiol in infusion solution by Q3 negative scanning mode.

As a result of scanning and optimizing the compound dependent parameters, a MRM method (Multiple Reaction Monitoring) was developed as a further step to develop a LC/MS/MS method. This method includes also the LC system with the parameters presented earlier. The response of the system to the new MRM method using a solution of 100 ng/ml in A/B 50:50 v/v was presented in figure 4.

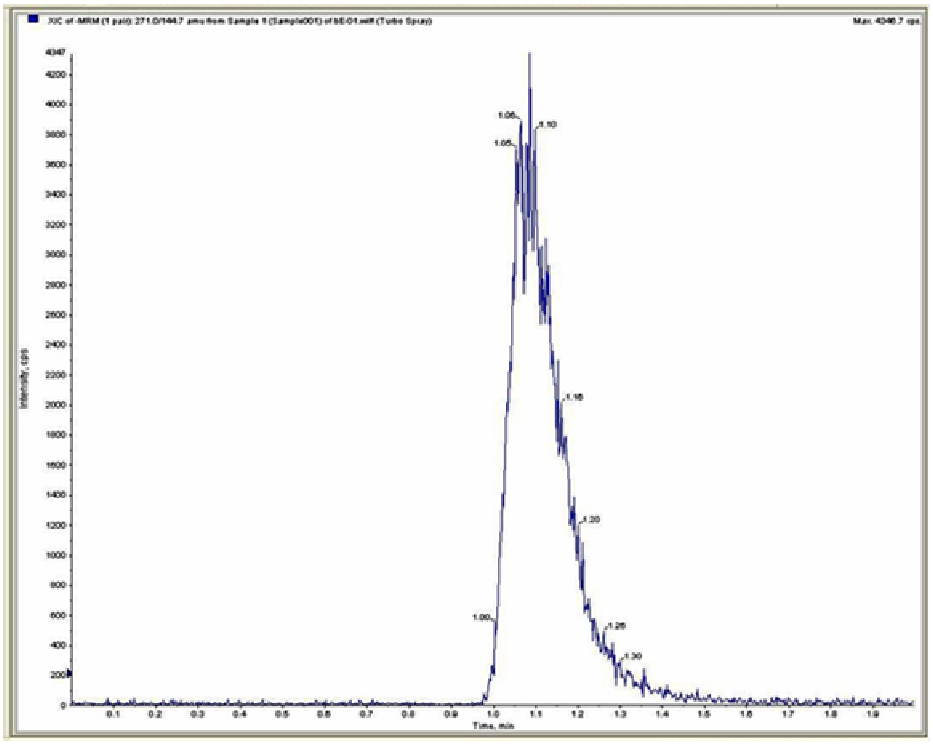

Fig.4. Chromatogram obtained for 100ng/mL 17β-estradiol in FIA solution by MRM method following the 271.1→144.8 transition

In order to obtain the appropriate mobile phase, using literature studies [1,9] different proportion of A and B were studied starting with A/B 50:50 v/v up to 100% A and then up to 100% B. Two injections were performed for every combination of A and B with a 0.2 mL/min flow in a 20 min scanning time. The best mobile phase was indentified as A/B 10:90 v/v.

Analyst program allows the automatic MS/MS optimization of the source dependent parameters using the selected mobile phase, FIA solution and a restriction column. After FIA analysis the following values of the source dependent parameters were obtained:

CUR (Curtain Gas): 18 psi;
IS (IonSpray Voltage): -2800V;
TEM (Teperature): 330°C;
GS1 (Nebulizer Gas): 18 psi;
GS2 (Turbo Gas): 18 psi:

The next step consisted in HPLC column selection. From 3 available columns, Phenomenex Luna C18 3µm, 50×2.0 mm, Phenomenex Gemini 3µm, 50×3.0 mm and Phenomenex Luna Phenyl-Hexyl 3µm, 50×3.0 mm, Phenomenex Luna C18 3µm, 50×2.0 mm proved to be more apropriate in the scanning conditions mentioned above, due to the shape and reproductibility of the chromatographic peak.

The column did not need any special cleaning on the process, only at the end of the day for safety porposes.

In order to verify the developed method a calibration curve was made in the range of 624 – 9980 ng/mL using diluted solutions from FIA solution. A calibration curve with a correlation factor of r=0.9979 was obtain using a linear regression function (1/x·x). The calibration curve is presented in figure 5 and the data results are presented in table 1.

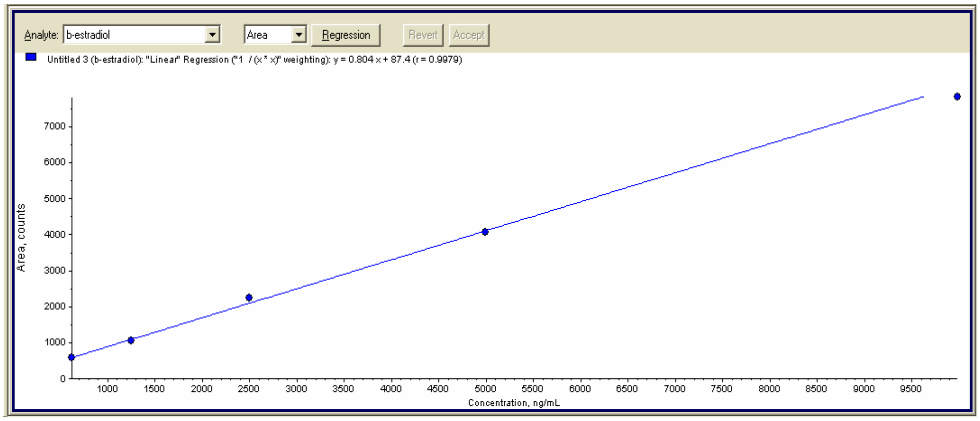

Fig.5. 17β-estradiol calibration curve following 271.1→144.8 transition

Tabel 1

Data results for 17β-estradiol calibration curve following 271.1→144.8 transition

Sample Name	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Analyte Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy (%)
1	7.83e+003	9.54e+002	9980	10600	106
2	4.06e+003	5.09e+002	4990	4330	86.7
3	2.26e+003	2.50e+002	2490	2700	108
4	1.05e+003	1.20e+002	1250	1520	109
5	5.90e+002	7.65e+001	624	619	99.2

One water sample was extracted using the sample preparation method mentioned above and studied with the developed method. The results showed the presence of the 17β-estradiol and its area was well fitted in the calibration curve and its concentration was determinate with the developed method.

During method development, the influence of the environmental conditions on the stability of the solutions was observed. In order to avoid their decomposition, all prepared solution were kept in dark glass recipients at -20ºC.

CONCLUSIONS

A rapid, sensitive and reliable method for quantitation of 17β-estradiol in waste water was developed using a 3200 QTRAP LC/MS/MS system with a TurboV in an ESI negative (electrospray ionization) mode. Specific compound and source dependent parameters were developed for MS instrument. Parameters for LC instrument were also established. A special interest was given to the flow rate, mobile phase and column selection.

A calibration curve was selected based on bibliographic studies and the method has been proved to have satisfying results on real waste water sample.

This method will be extended in future in order to obtain even lower detection limit and a better recovery. Also, its extinction for simultaneous analysis of multiple estrogens from water is expected.

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