*Supplementary information*

# Experimental Design and Bioassays as Tools to Investigate the Impact of Anodic Oxidation on Progestins Degradation

## *1. Supplementary figures*

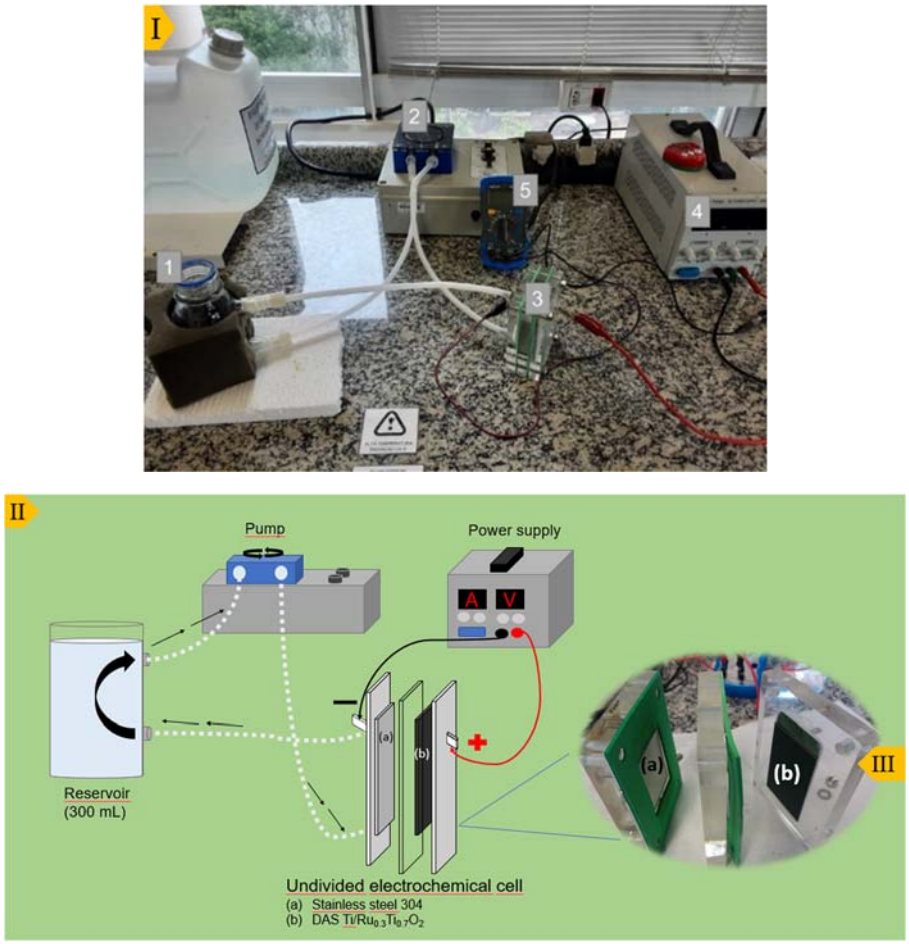



**Figure S1 –** (I) Electrochemical apparatus used for the degradation of progestogens via anodic oxidation: (1) reservoir; (2) peristaltic pump; (3) electrochemical cell; (4) power supply; (5) multimeter. (II) Schematic representation of the electrochemical apparatus. (III) Disassembled electrochemical cell.

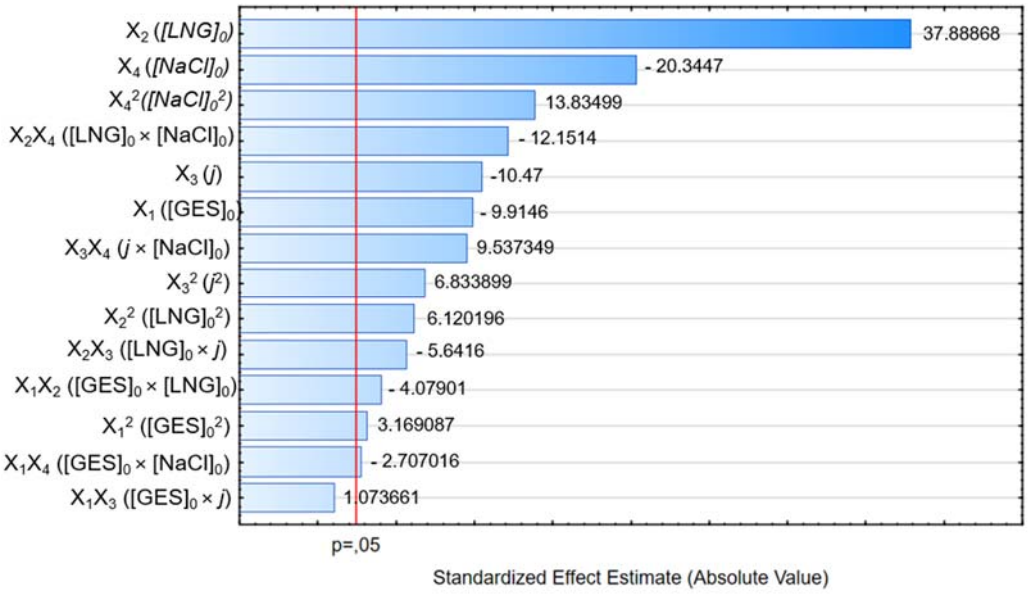



**Figure S2** – Pareto chart of standardized effects.

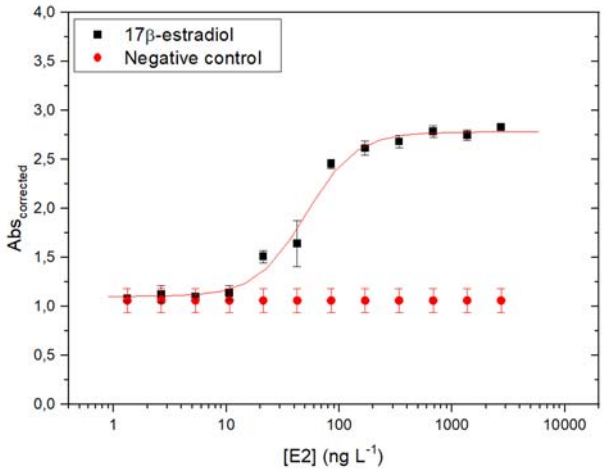



**Figure S3** – Dose-response curve for the positive control E2 (acetonitrile: water of 55:45 v/v. LOD = 2.3 ng $L^{-1}$ and LOQ = 7.0 ng $L^{-1}$).

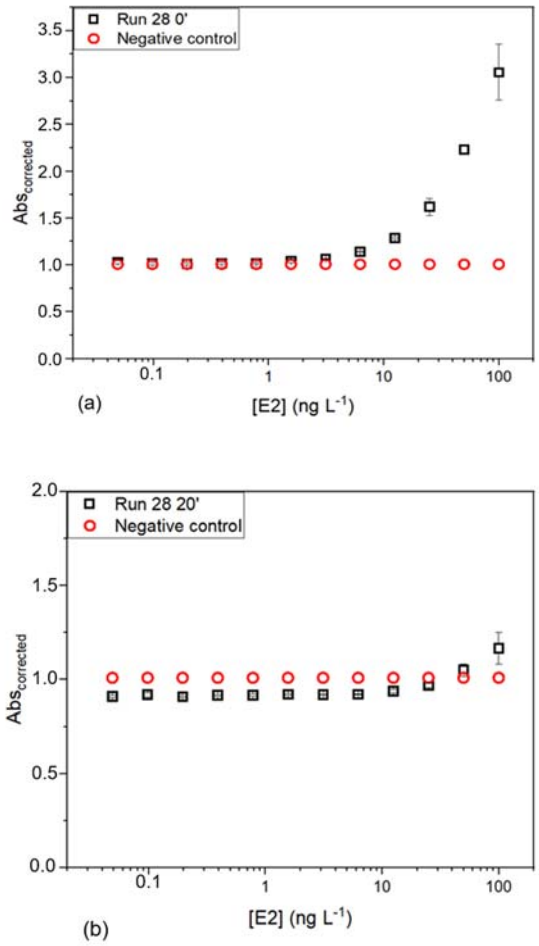
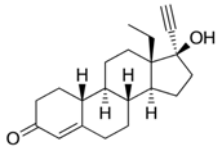
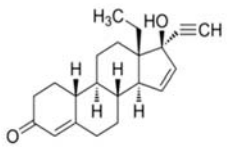



**Figure S4** – Dose-response curve for experiment 28 before (a) and after (b) the anodic oxidation process ($[NaCl]_0$ = 0.07 mol $L^{-1}$, $j$ = 32.5 mA $cm^{-2}$, $[LNG]_0 = [GES]_0$ = 1.0 mg $L^{-1}$).

## *2. Supplementary tables*

**Table S1** – Physical and chemical properties of progestins under study.

| Progestins | Chemical structure | Molar weight (g $mol^{-1}$) | Water solubility (25 °C) (mg $L^{-1}$) | $UV_{maximum}$ (nm) | $pK_{ow}$[a] | $pK_{a1}$ |
|---|---|---|---|---|---|---|
| Levonorgestrel |  | 312.4 | 2.05 | 241 | 3.48 | 17.91 |
| Gestodene |  | 310.4 | 8.12 | 244 | 3.26 | 17.08 |

[a]Octanol-water partition coefficients at 20 °C.

**Table S2** – Validation parameters of the LNG and GES calibration curves obtained by UFLC analysis. LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation; CI: confidence interval.

| Progestin | LOD (mg $L^{-1}$) | LOQ (mg $L^{-1}$) | $R^2$ | Recovery (%) | RSD* (%) | CI** |
|---|---|---|---|---|---|---|
| LNG | 0.02 | 0.07 | 0.9991 | 91.60 ± 0.04 | 24.0 | 0.33 ± 0.10 |
| GES | 0.06 | 0.20 | 0.9980 | 84.90 ± 2.47 | 33.0 | 1.40 ± 0.06 |

* The relative standard deviation (RSD) corresponds to the quotient between the standard deviation ($s$) and the mean value ($\bar{x}$) of $n$ = 30 independent measurements (replicates) of LNG and GES concentrations in solution, multiplied by 100. ** The confidence interval for the mean is given by $CI = \bar{x} \pm t_{n-1} \frac{S}{\sqrt{n}}$, where $t_{n-1}$ corresponds to the critical value of Student's t distribution with $n-1$ degrees of freedom and 95% confidence level (Ribani, M.; Bottoli, C. B. G.; Collins, C. H.; Jardim, I. C. S. F.; Melo, L. F. C. Validação em Métodos Cromatográficos e Eletroforéticos. Quimica Nova 2004, 27, 771–780, doi: 10.1590/S0100-40422004000500017).