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Solid-surface fluorescent properties of estrogens: Green analytical applications

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ABSTRACT

For the first time, the fluorescent signals produced by 17β-estradiol (E2), estradiol valerate (E2V) and 17α-ethinylestradiol (EE2) were investigated after being retained in selected solid surfaces. Among different studied supports, both C18 and nylon membranes demonstrated to have been able to induce intense signals over their surface, after aqueous solutions of the analytes were retained via a solid-phase extraction procedure. Physicochemical variables which affect the quality of the fluorescence signals were optimized, and the results obtained in the studied solid supports were compared and discussed. While nylon successfully faced the trouble of spectrofluorimetrically quantifying estrogens in pharmaceuticals with unbalanced estrogen/progestagen ratios, C18 membranes showed very good qualities for second-order spectrofluorimeric measurements, allowing the determination of E2 (the most active estrogen) in potentially contaminated fish and chicken samples. The fluorescence excitation–emission matrices, directly measured on a C18 surface, were processed using appropriate chemometric algorithms in order to efficiently quantify E2 in the presence of natural matrix constituents. The present strategy avoids the elution step, considerably decreasing the use of organic solvents, the analysis time and the experimental errors. In addition, the instrumental involved is nonsophisticated and, therefore, the experiments could be carried out in routine laboratories.

1. Introduction

Estrogens are steroidal sex hormones produced by the ovaries, the placenta during pregnancy and in smaller amounts by the adrenal glands. These hormones are usually prescribed in replacement therapies, for the treatment of menopausal symptoms, and for contraception to prevent pregnancy. Both the natural 17β-estradiol (E2) and the synthetic estradiol valerate (E2V) are the main estrogens applied in hormonal replacement therapy [1]. On the other hand, the synthetic estrogen ethinylestradiol (EE2) and progestagens of different generations are present in most oral contraceptives.

While estrogens exhibit weak fluorescence in aqueous solution, more intense signals are obtained in organic solvents and, thus, most current spectrofluorimetric methods for estrogen quantification are developed in the latter media. Although several decades ago the fluorimetric determination of estrogens was usually based on the signals produced in hot concentrated sulfuric acid [2], due to these drastic experimental conditions, the latter practices became unpopular. As part of a plan committed to the development of green analytical methods, the present paper evaluates the fluorescent properties of selected estrogens adsorbed in solid surfaces, in order to assess the usefulness of the signal for analytical purposes. To the best of our knowledge, this is the first report about the fluorescence signals produced from estrogens adsorbed on solid supports.

Solid-surface fluorescence (SSF) analysis is very sensitive for the determination of a great variety of organic compounds, and is especially valuable for the development of green analytical techniques [3]. In fact, with the purpose of demonstrating the usefulness of SSF for the development of green methods, two novel strategies for estrogen determination in different types of samples are implemented.

One of them involves the use of a nylon membrane as a support for a solid-phase extraction (SPE) procedure, combined to univariate fluorescence calibration for estrogens determination in various commercial pharmaceuticals, with especial interest in those containing a significantly large amount of progestagens in relation to the estrogen content. Progestagens do not emit natural fluorescence, but produce a serious interference through the inner filter effect, if they are simultaneously retained with the estrogen in the solid support.

In a second example, the most active estrogen E2 is retained from both fish and chicken tissue extracts on a C18 membrane, and is then determined by excitation–emission fluorescence matrices (EEFMs), directly recorded on the surface of the solid substrate. Subsequently, three chemometric algorithms which achieve the second order advantage, namely, parallel factor analysis (PARAFAC) [4], unfolded partial least-squares coupled to residual bilinearization (U-PLS/RBL) [5], and multivariate curve resolution-alternating least-squares (MCR-ALS) [6] are applied to process the EEFMs, and their prediction capabilities are

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discussed. The term "second-order advantage" refers to the capacity of certain second-order algorithms to predict concentrations of sample components in the presence of any number of unsuspected constituents [7]. All results obtained with the proposed strategies are discussed and compared with those provided by a chromatographic reference method.

2. Materials and methods

2.1. Reagents and solutions

All reagents were of high-purity grade and were used as received. E2, E2V, EE2, levonorgestrel (LNG), desogestrel (DSG), norgestimate (NGM), norethisterone acetate (NTA) and cyproterone acetate (CTA) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Methanol (MeOH) and acetonitrile (ACN) were obtained from Merck (Darmstadt, Germany), while trichloroacetic acid (TCA) was obtained from Anedra (San Fernando, Argentina).

Different lots of nylon membranes of 0.2 μm pore size were tested: Varian (Seattle, USA), Scleicher-Schuell (Dassel, Germany), GE Osmonics (Trevose, USA), and Whatman (Sigma-Aldrich, USA). No significant differences were observed among these membranes, either in the background emission or in the luminescence properties of the retained estrogens. Two C18 solid-phase-extraction disks were tested: ENVI-18 DSK and Empore Octadecyl C18, both purchased from Supelco (Bellefonte, PA, USA). The surface-modified silica is contained in a glass fiber matrix in the former disks and in Teflon in the latter ones. Although the obtained signals are similar in both disks, the experiments were carried out using ENVI-disk because they are thicker, more rigid and less expensive than disks based on Teflon.

MeOH stock solutions of E2, E2V and EE2 of about 2500 μg ml^{-1} were prepared and stored in dark flasks at 4 °C. From these solutions, more diluted methanol solutions were obtained. Working aqueous solutions were prepared immediately before their use by taking appropriate aliquots of methanol solutions, evaporating the organic solvent by use of dry nitrogen and diluting with ultrapurified water from a Millipore system (Massachusetts, USA) to the desired concentrations.

2.2. Instrumentation

Fluorescence measurements were done on an Aminco Bowman (Rochester, NY, USA) Series 2 luminescence spectrometer equipped with a 7 W pulsed xenon lamp. Specific wavelengths for the different analyses will be discussed below. The slit widths were both of 4 nm. All fluorescence measurements were performed at 20 °C using the thermostated cell holder and a Lauda (Frankfurt, Germany) RM6T thermostatic bath. The pH of solutions was measured with a Metrohm (Herisau, Switzerland) 713 pH meter equipped with a combined glass electrode. Absorbance data were obtained with a Beckman 120 (Fullerton, CA, USA) DU 640 spectrophotometer. High-pressure liquid chromatography (HPLC) was carried out on an Agilent 1200 (Agilent Technologies, Waldbronn, Germany) liquid chromatograph with an Agilent 1260 Infinity diode array detector. The system was controlled by the ChemStation software.

2.3. SPE-solid-surface fluorescence

The employed 47-mm C18 membranes were dissected into 13-mm disks. The disk was loaded into a stainless steel filter syringe kit (Alltech, Deerfield, IL, USA), and was conditioned with 0.5 ml of MeOH followed by 0.5 ml of water. With the purpose of concentrating the analyte into a restricted area of the solid surface, a Teflon ring was fitted over the membrane before the extraction. Thus, an extractive surface with final diameter of 5 mm was exposed to the flowing solution. A 5 ml syringe was coupled to the filter holder and positive pressure was used to force the solution through the membrane in approximately 30 s per sample. This flow rate is in the optimum range for maximum breakthrough volume (10–30 ml min^{-1}) [8]. Following the estrogen extraction, the sample was partially dried by forcing air through the disk using a 25 ml syringe. The disk was then removed from the holder, it was placed in a laboratory-made SPE membrane holder [8] and the fluorescence spectra were measured. The angle formed between the excitation and emission beams was 90°, with an incident angle of 45°.

The procedure performed with nylon membranes was similar to that carried out with C18, but in this case the membrane did not need conditioning with MeOH. Before the extraction procedure the nylon disk was dried on a heating plate for 10 min at about 100 °C.

2.4. Zeroth-order calibration for pharmaceutical analysis

E2, E2V and EE2 solutions for the calibration curves were obtained by performing convenient dilutions of standard aqueous solutions with water. The former were prepared from the corresponding MeOH standards. Then, SPE (both C18 and nylon) and fluorescence measurements were carried out by the procedure described above, setting the excitation and emission wavelengths at 280 and 310 nm, respectively.

The analyzed pharmaceutical preparations were Lindisc 50 (Bayer, Buenos Aires, Argentina), Rontagel (Ferring, Argentina), Ciclocur (Bayer, São Paulo, Brazil), April (Gador, Buenos Aires, Argentina), Marvelon (Organon, Buenos Aires, Argentina), Evra (LTS Lohmann Therapie-Systeme AG, Andernach, Germany), Cilest (Cilag International, Zug, Switzerland), Diane 35 (Schering, Buenos Aires, Argentina), Primosiston (Bayer, São Paulo, Brazil), and Ginelea (Elea, Montevideo, Uruguay).

2.4.1. Estradiol and ethynylestradiol (tablets)

Ten tablets of the corresponding preparation were weighed in order to find the average tablet mass, triturated and mixed. Alkaline solutions of each commercial sample were prepared by weighing about 100.0 mg of the latter mixture, treating them with 3.00 ml of NaOH solution (final pH ≈ 13) and stirring the mixture during 20 min. The mixture was then centrifuged for 10 min at 12,000 rpm. Aliquots of 200 or 250 μl of supernatant were treated with HCl and water in order to obtain an aqueous solution of pH about 7 and 5.00 ml of final volume.

2.4.2. Estradiol (patch)

One square centimeter of Lindisc 50 patch was treated with 1.50 ml of methanol, sonicated during 10 min, and centrifuged during additional 10 min. An aliquot of 200 μl of supernatant was diluted with 1.00 ml of MeOH. From this latter solution, an aqueous solution was prepared by taking an aliquot of 100.0 μl, evaporating the solvent with nitrogen, and diluting with water to 5.00 ml.

2.4.3. Estradiol (gel)

A mass of 0.400 g of gel was dissolved in 10 ml of methanol, sonicated during 10 min, and centrifuged during additional 10 min. An aqueous solution was prepared by taking 100 μl of supernatant, evaporating the solvent with nitrogen, and diluting with water to 5.00 ml.

2.4.4. Estradiol valerate

Ten tablets of Ciclocur (brown pills) were weighed, triturated and mixed, and the same procedure was separately carried out with 10 tablets of Ciclocur (white pills). An amount of 20.0 mg of the mixture was treated with 1.00 ml of MeOH and sonicated by 10 min. The mixture was then centrifuged, and 100 μl of the supernatant was diluted with 1.00 ml of MeOH. From this latter solution, an aqueous solution was prepared by taking an aliquot of 50.0 μl, evaporating the MeOH by use of nitrogen and diluting with water to 5.00 ml.

2.4.5. Ethynylestradiol (patch)

One square centimeter of Evra patch was treated with 1.00 ml of MeOH, sonicated during 10 min, and centrifuged during additional