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Application of hybrid mesoporous silica for extraction of hormones in milk by matrix solid phase dispersion

J. Gañan, M. Silva, S. Morante-Zarcero, D. Pérez-Quintanilla, I. Sierra *

Departamento de Química Inorgánica y Analítica, E.S.C.E.T., Universidad Rey Juan Carlos, 28933 Móstoles (Madrid), Spain

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ABSTRACT

A matrix solid phase dispersion (MSPD) method for simultaneous determination of five natural and synthetic steroids in goat milk was proposed. SBA-15 type mesoporous silica were functionalized with octadecylsilane, and the resulting hybrid material (SBA-15-C18) was applied as a solid support for MSPD. The synthesized material showed high affinity to steroids in milk, and the obtained extract was sufficiently cleaned to be directly analyzed by micellar electrokinetic chromatography (MECK). The proposed method was validated in terms of linearity, precision, accuracy, limit of detection and quantitation. To the best of our knowledge this is the first application where hybrid mesoporous silica have been used to determine hormones in milk by a MSPD–MECK method.

1. Introduction

The alarming presence of emerging contaminants in food has increased dramatically and makes the concern for quality and food safety. A very worrisome of these pollutants are steroid hormones that are found in food naturally, but can also accumulate due to illegal use as growth promoters in animals. Testosterone (T), progesterone (P), estrone (E1), 17β-estradiol (17β-E2) and ethinylestradiol (EE2) are certain of these compounds. The administration of anabolic growth promoters in animals is now prohibited because of their potential risk to human beings [1]. According to scientific studies these hormones have been associated with the dramatic increase in breast cancer and uterine cancer in females and testicular cancer in men, even at very low concentration levels [2].

Concerned about the quality and safety of food, the objective of this work has been the development of a new simple method for the determination of steroids in milk combining matrix solid phase dispersion (MSPD) and micellar electrokinetic chromatography (MECK). The use of MSPD offers a simple preparation, reduces the use of extraction solvents and length of sample preparation. This methodology allows the complete disintegration and dispersion of the sample on a solid support, thereby generating a solid mixture having the chromatographic character sufficient to extract the analytes [3].

Recent advances in the development of new materials are having a major impact on Analytical Chemistry [4]. In that respect, the sorbent used in the MSPD procedure developed in this work was SBA-15 type mesoporous silica, with uniform ordered structure, high surface area, and high pore volume. Likewise, chemical modification of this material with octadecylsilane groups allowed obtaining hybrid mesoporous silica (SBA-15-C18) capable of adsorbing 17β-E2. The retention mechanism is primarily governed by hydrophobic interactions between the analyte and the carbonaceous moieties of the alkyl chain [5]. In the present paper, SBA-15-C18 was evaluated for the first time as sorbent in MSPD. The method presented was rapid, simple and efficient for the determination of T, P, E1, 17-β-E2 and EE2 in goat milk by MECK.

2. Experimental

Preparation of mesoporous silicas: Mesoporous silica (SBA-15 type) were prepared according to the method of Zhao et al. [6]. A post-synthesis method was used to functionalize the material, in order to obtain SBA-15-C18, by reaction of 12 g of SBA-15 with 1.74 g of chloro(dimethyl)octadecyl silane dissolved in 50 mL toluene. The mixture was heated at 80 °C for 24 h at 500 rpm. Finally, the solid was washed with two fractions of 50 mL of toluene, ethanol, and ethylic ether.

Characterization of mesoporous silicas: X-ray diffraction patterns of silicas were obtained on a Philips diffractometer model PW3040/00 X'Pert MPD/MRD at 45 kV and 40 mA, using Cu Kα radiation ($\lambda = 1.5418$ Å). Scanning electron micrographs and morphological analysis were carried out on a XL 30 ESEM Philips with an energy dispersive spectrometry system. Conventional transmission electron microscopy was carried out on a TECNAI 20 Philips, operating at 200 kV. N_2 gas adsorption–desorption isotherms were

* Corresponding author. Tel.: +34 914887018.
E-mail address: isabel.sierra@urjc.es (I. Sierra).

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obtained using a Micromeritics ASAP 2020 analyzer, and pore size distributions were calculated using the Barrett–Joyner–Halenda (BJH) model on the adsorption branch. Elemental analysis (%C) was performed with a LECO CHNS-932 analyzer (Universidad Complutense de Madrid, Spain). The thermal stability of the modified mesoporous silicas was studied using a Setsys 18A (Setaram) thermogravimetric analyzer (from 25 to 800 °C at 10 °C/min).

Hybrid mesoporous silica-based matrix solid phase dispersion extraction procedure: 200 μL of fortified milk sample was placed into a glass mortar and gently blended with 0.100 g of SBA-15-C18, 0.100 g of Na_2SO_4 and 0.100 g of washed sea sand by using a glass nail until a dry and homogeneous mixture was obtained. Then, the mixture was packed into a SPE cartridge with a plug, with porous PTFE disks at both ends that retain the entire mixture. The cartridge was washed with 1 mL of hexane and then the targeted analytes were eluted using 3 mL of methanol, at a flow rate of 1 mL/min. Then the eluent was dried using a vacuum line, and the residue was reconstituted with 500 μL of MeOH/H_2O (50:50, v/v) and filtered through 0.45 μm pore size disposable nylon filters.

Micellar electrokinetic chromatography determination: Separations were performed using a Beckman P/ACE MDQ CE system on untreated fused-silica capillaries (50 μm ID, 362.1 μm OD) purchased from Polymicro Technologies (Phoenix, AZ, USA). Capillaries had a total length of 60.2 cm and 50.0 cm when measured by the detector. The detection was performed at 249 nm for T and P, and at 200 nm for E1, 17β-E2 and EE2, with a band width of 10 nm. The running background electrolyte (BGE) was 25 mM borate, 10 mM sodium dodecyl sulfate and 20% acetonitrile. The selected instrumental conditions were capillary T^a, 15 °C; injections by pressure, 0.5 psi × 3 s of sample followed by a plug of 0.1 psi × 1 s of BGE, and applied voltage, 27 kV.

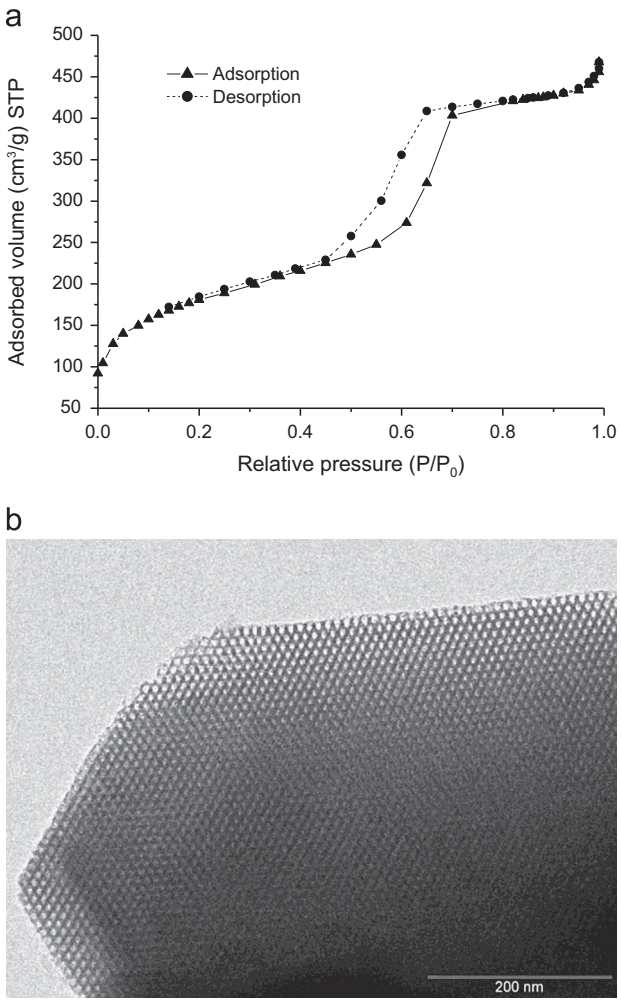

Fig. 1. (a) N_2 adsorption–desorption isotherms of SBA-15-C18. (b) TEM micrograph of SBA-15-C18.

3. Results and discussion

Mesoporous silica characterization: XRD patterns of the synthesized materials showed that they display well-resolved patterns at low 2θ values, with a very sharp (100) diffraction peak around 0.9 Å and two well-resolved weak diffraction peaks (110) at 1.5 Å and (200) at 1.8 Å. These data suggest a significant degree of long range ordering of the structure and well-formed 2D hexagonal pore arrays. N_2 adsorption–desorption isotherms for SBA-15 and SBA-15-C18 were of type IV, according to the I.U.P.A.C. classification, with an H1 hysteresis loop that is representative of materials with pores of constant cross-section (Fig. 1a). In SBA-15, the Brunauer–Emmett–Teller specific surface area (S_{BET}), pore volume and a BJH (Barrett–Joyner–Halenda) pore diameter of this material were 830 m^2/g, 0.80 cm^3/g and 64.2 Å, respectively. After functionalization, the obtained SBA-15-C18 material possessed lower S_{BET} (646 m^2/g), pore volume (0.68 cm^3/g) and BJH pore diameter (63.7 Å). TEM micrograph of both materials demonstrated a clear arrangement of hexagonal pores with uniform size (Fig. 1b) and SEM micrographs showed a uniform particle size with cylindrical shape. The quantity of C18 groups attached to the mesoporous silica, calculated from the %C obtained by elemental analysis, was 0.26 mmol/g. TGA of SBA-15-C18 showed that an exothermic degradation process occurred between 200 and 600 °C with a weight loss around 5%. The thermal stability of this material is in agreement with previous results given in the literature for other functionalized mesoporous silicas and confirms the quantity of C18 groups estimated by elemental analysis.

Determination of hormones: The first step in the method setup was the evaluation of a suitable mixture to allow complete adsorption of matrix components and to facilitate the transfer into the MSPD cartridge. In this study, different ratios were evaluated using 200 μL of milk spiked with the mixture standard of steroids, in order to obtain a concentration of 50 μg/mL. Finally, using a ratio 2/1/1/1 (sample/SBA-15-C18/sea sand/Na_2SO_4), a homogeneous and dry mixture was obtained that allowed the easy packing in the cartridge and also the flow of solvents through it. On the other hand, an appropriate washing solvent should leave the target compounds adsorbed on the cartridge and remove matrix interferences from the sample as much as possible. Then, 1 mL and 2 × 1 mL of hexane and 0.5 M of NaOH were investigated

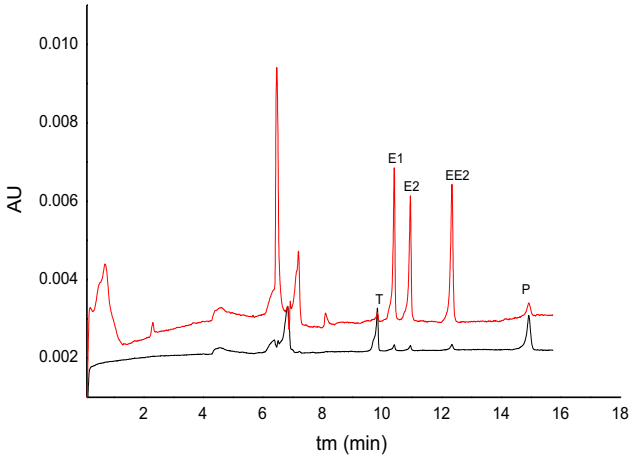

Fig. 2. Typical electropherogram of a spiked goat milk sample treated under the optimized MSPD conditions. For electrophoretic conditions see Section 2.