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Use of Sugar Cane Bagasse as Solid Extraction Phase Sorbent to Analyze Hormones from Industrial Effluent

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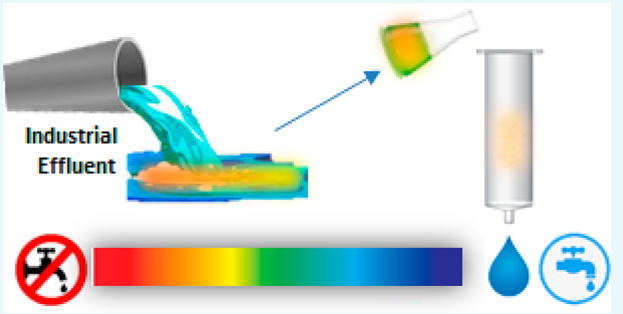
ABSTRACT: Sugar cane bagasse (SCB) is an abundant byproduct of sugar and bioethanol mills. It has been effectively used as a low-cost biosorbent to remove hazardous chemical compounds from a variety of effluent sources. Herein, we report on the preparation of SCB and its use as a solid phase extraction (SPE) sorbent to retain synthetic hormones (ethinylestradiol, drospirenone, and levonorgestrel) from industrial pharmaceutical plant effluent samples prior to LC−MS/MS quantitative analysis. We evaluated the reproducibility and recoveries and accuracy data analyses were compared with that of commercial SPE (cSPE) cartridges. The results from the evaluated parameters indicated that the SCB bed had an efficiency of >99%, comparable to that of cSPE cartridges, demonstrating the applicability and feasibility of this material as an effective and green chemistry alternative, as well as its biosorbent potential to remove hormones from industrial pharmaceutical effluent.



INTRODUCTION

For more than three decades, the solid phase extraction (SPE) technique has been utilized as an analytical tool for rapid, selective, sample cleanup and purification, as well as for prior qualitative or quantitative trace analysis of organic compounds.[1−5] SPE is a technique comparable to liquid chromatography in that both techniques use the same stationary phase (sorbent) and mobile phase (extraction solution). These techniques are all based on the same analytical concept and goal: the separation of the compounds of interest present in the samples without interferences through selectivity or interaction, between the phases (solid and liquid). Thus, the effectiveness of SPE is dependent on the specificity of the sorbent material and its physicochemical characteristics of extracting, partitioning, and/or trapping organic compounds from complex materials such as biological, environmental, and food matrices.[6−9] The challenge of developing a novel SPE sorbent is based on the following parameters: (a) high extraction capacity (mass transfer); (b) increased selectivity or specificity; (c) high chemical and thermal stability; (d) amelioration of compatibility with complex samples; (e) increased environmental sustainability; and (f) cost-effectiveness as described by Pedersen-Bjergaard and Hasen in their published review.[10] Additionally, the authors demonstrated a trend toward the investigation of novel materials for use as SPE sorbents (e.g., molecularly imprinted polymers, metal−organic frameworks, covalent organic frameworks, carbon-based sorbents, graphene and graphene oxide, restricted access materials, immunosorbents, monoliths, zeolites, and metallic nanoparticles). Since 2016, as noted in the published review by Płotka-Wasylka et al.[11] the SPE sorbent materials cited here have been studied and explored for a variety of various classes of organic compounds and matrices. The purpose of this present study is to investigate and use sugar cane bagasse (SCB) as a biosorbent using green chemistry principles in order to increase the quality of the results while being ecologically benign. SCB is a fibrous material remaining after the plant's stalk pressing process, used to extract the sweet juice used for the industrial production of sugar and alcohol. Furthermore, SCB is one of the most economically viable and readily accessible agro-industrial residues in the world, particularly in tropical regions.[12] Brazil is the world's largest producer of sugar cane (*Saccharum* sp.), followed by India, China, and Bangladesh,[13] all of which generate significant amounts of bagasse as a byproduct of their sugar and bioethanol mills in each of these countries. Additionally, SCB exhibits strong biosorption capabilities, which are defined as the passive sorption of organic and inorganic substances in soluble or insoluble forms from an aqueous solution utilizing decomposing biological materials. Sarker et al.[14] published a comprehensive study of the SCB biosorption properties and their application. The authors of

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that study discuss the use of SCB to remove chemical contaminants from aqueous (waste) solutions, including heavy metals;[15,16] dyes;[13] petroleum;[17] phenolic compounds[18] and organic nutrients.[19] The purpose of this work is to demonstrate the continued applicability of SCB as an SPE biosorbent bed for extracting synthetic hormones (an emerging environmental contaminant referred to as endocrine-disrupting chemicals (EDCs) from pharmaceutical industrial effluent prior to LC−MS/MS quantitative analysis. The major contraceptive synthetic hormones such as ethinylestradiol, drospirenone, and levonorgestrel are persistent micropollutants that enter the water systems mostly through domestic sewage and untreated industrial effluent pose detrimental effects on living organisms, wreaking havoc on their endocrine systems. These EDCs are considered toxic to aquatic microorganisms causing negative consequences such as feminization, infertility, reproductive difficulties, and genital abnormalities, and hence have a direct effect on the ecology. In humans and animals, EDCs are associated with certain adverse health effects including decreased sperm counts in adult males and an increase in hormone-dependent cancers (e.g., breast or prostate cancer). Additionally, EDCs are notoriously difficult to remove from industrial and municipal effluents (via aerobic and anaerobic methods). The European Water Framework Directives on Priority Substances includes these synthetic hormones as emerging contaminant of concern as they have been detected and quantified at low concentrations in natural water at the pg/L levels.[20−22] At low concentrations these EDCs can be 10 to 50 times more potent, and their long half-life makes them more available for bioaccumulation in key marine ecosystems and their associated microbiomes. The SCB can be used as an alternative and advantageous material in the analytical chemistry applications utilizing SPE, as well as in the treatment of industrial and domestic effluents. The following factors prompted this work: (a) well-defined SCB physicochemical characteristics from the presence of macromolecules (e.g., humic and fulvic acid, lignin, cellulose, hemicelluloses, and proteins) that contain various functional groups responsible for the absorption sites, including phenol (−OH), carboxylic (−COOH), amine ($-NH_2$), amide ($-CONH_2$), and hydrogen sulfite ($-SH_2$);[23] (b) the absorption processes involving ion exchange (attraction and substitution of ions from the organic compounds by hydroxonium H_3O^+ ions) and/or complexation at the accessible SCB binding sites (donation of the electron pair). Moreover, the presence of cellulose and lignin (biological polymers) enhances absorption capabilities via their polarity and chemical affinity, as well as by arranging the morphological surface of the bagasse;[24−26] (c) the abundant material derived from biomass; and (d) the application of green chemistry. The sorption effectiveness of the SCB bed was evaluated using the following experimental parameters in comparison to current and commercially available SPE (cSPE) cartridges: (i) SCB reuse (recycling); (ii) load concentration variation; (iii) breakthrough (mass/volume capacity); (iv) pH variation; (v) extraction solvents variation; (vi) particle size; and (vii) suitability for use in industrial effluent.

EXPERIMENTAL SECTION

Chemicals and Reagents. The chemical standards of ethinylestradiol (99.6% purity), levonorgestrel (99.6% purity), and drospirenone (99.8% purity) were acquired from the local pharmaceutical industry (São Paulo, Brazil). Estradiol utilized as an internal standard (control) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Each compound was prepared as stock solutions at a concentration of 1 mg/mL in methanol. LC−MS grade acetonitrile was purchased from Supelco (Darmstadt, Germany), HPLC grade acetone (J. T. Baker, Center Valley, PA), ACS grade ethanol (J. T. Baker, Mexico), ACS grade ethyl acetate (Macron, Mexico), and formic acid (<98%) for mass spectrometry were procured from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol was obtained from Merck (Darmstadt, Germany), and HPLC grade *n*-hexane and isopropyl alcohol were purchased from Tedia (Fairfield, OH, USA). At last, Na_2HPO_4, NH_4HCO_3 salts, HCl (37%), and NaOH (<98%) were purchased from the local Sigma-Aldrich (São Paulo, Brazil).

Sugar Cane Bagasse (SCB) Bed Preparation. The peeled triturate sugar cane tales were collected from a local farmer's market and washed with Milli-Q water 18.2 mOms (Integral 3, Millipore, France) to remove juice residues and impurities. Then, the tales were dried for 48 h at 50 °C inside the Fanem Orion 515 drying oven (Fanem, São Paulo, Brazil). Approximately 250 g of the dried tale was triturated in small pieces using a semi-industrial blender. Fifteen grams of the material was retriturated for 24 h in a laboratory ball mills apparatus (Retsch, GmbH, Germany) to create a fine SCB powder. The SCB powder was filtered using molecular sieves of 2 mm, 1 mm, and ultimately 75 μm. Until use, this material was kept at room temperature in a laboratory desiccator chamber containing silica balls.

Sample Preparation-Industrial Effluent. Four samples were obtained from a pharmaceutical plant located in Sao Paulo, SP, Brazil, before its treatment. These samples were collected over a period of several months to monitor charge/concentrations. The viscosity of the samples was high, and the samples were orange in color. A pool of these samples was prepared as follows: samples were homogenized; 125 mg of each sample was weighed and diluted in 50 mL of distillated water (18.2 mOms) at a concentration of 10 mg/mL w/v (1:100 dilution). Prior to the SPE loading procedure, the samples were vortexed and filtrated using a 0.45 μm PTFE (Millipore, France) syringe filter. This sample pool was applied in replicates (n = 6) to evaluate the precision and others SPE parameters.

Solid Phase Extraction (SPE). An SCB 75 μm bed at a concentration of 50 mg was used to pack empty 1 cc (mL) SPE cartridges containing retained frits (Supelco, Inc., PA, USA). To compare the outcomes of the recoveries, the following cSPE were used: Oasis HLB 30 mg, 1 mL (Waters, MA, USA); Discovery DSC-18 50 mg, 1 mL, and Sulpeclean Envi-Carb, 100 mg, 1 mL (Supelco, Inc., PA, USA). A standard cSPE protocol procedure was applied: conditioning with 1 mL of methanol followed by 1 mL of water; load of 1 mL of sample; wash 2× with 0.75 mL of water; dry the cartridge under synthetic air flow; elution 2× with 0.3 mL MeOH. Visiprep 24 Supleco Inc. (PA, USA) apparatus was utilized to support the SPE experiment. Prior to LC−MS/MS analysis, the MeOH extracted sample residue was dried in a 10 mL class assay tube under N_2 gas flow at 40 °C using a sample concentrator dry-block DR-3D (Techne, UK) and dissolved in 1 mL of 50% acetonitrile/50% water (v/v) solution.

LC−MS/MS Analysis. The HPLC system (binary pump and degasser) with a refrigerator autoinjector and column oven, model series 1260 from Agilent Technologies (Santa Clara, CA, USA) equipped with a reversed-phase C_{18} analytical

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Table 1. Comparison Results from Different SPE Cartridges (Effluent Industrial Concentration and Accuracy[a])

	estradiol		ethinylestradiol		levonorgestrel		drospirenone	
SPE cartridges	concn (ng/mL)[b]	accuracy (%)	concn (μg/mL)	accuracy (%)	concn (μg/mL)	accuracy (%)	concn (μg/mL)	accuracy (%)
SCB 50 mg[c]	119	120	2.43	119	6.74	105	65.0	99.2
Oasis HLB 30 mg	99.1	100	2.04	100	6.40	100	65.5	100
DCS-18 50 mg	108	109	1.67	81.9	6.16	96.3	61.3	93.6
Envi-Carb 100 mg	N.D.	0.0	0.151	7.40	1.49	23.3	N.D.	0.0

[a]Accuracy compared with Oasis 30 mg. [b]Spiked concentration result from 100 ng/mL. [c]Concentration results from average of $n = 6$. N.D. = not detected.

column of 50 mm × 2.0 mm and a 4.0 μm particle size (Synergy Fusion-RP 80A, Phenomenex, USA) was used coupled with the mass spectrometer analyzer. The temperature of the column was maintained at 45 °C. The injected sample volume was 10 μL. Mobile phases A and B were water with 0.1% formic acid and acetonitrile, respectively. The optimized chromatographic method maintained the initial mobile phase composition (5% B) constant for 0.5 min, followed by a linear gradient to 90% B in 5.0 min and holding at this concentration for 3.0 min. The chromatographic run time was 8 min plus 2.5 min for initial conditional equilibration, for a total of 10.5 min with a flow rate of 0.4 mL/min for sample analysis. A hybrid triple quadrupole linear ion trap mass spectrometer (QqLIT) model 3200 QTRAP (Sciex, Concord, Canada) was utilized to quantify the compounds. The compounds were ionized using atmospheric pressure chemical ionization (APCI) operated in the positive mode at the following parameters: needle current (NC), 4 μA; temperature, 450 °C; heater gas (GS1), 40 psi; and curtain gas, 25 psi (with interface heater on). The multiple reaction monitoring (MRM) mode was utilized to quantitatively analyze the target compounds using the triple quadrupole stage, and the optimal signal transition conditions were as follows (the bold font highlights the MRM quantitation transitions followed by the confirmation): estradiol (control compound) 255 > 133 and **255 > 159**, declustering potential (DP) = 48 V, entrance potential (EP) = 7 V, collision entrance potential (CEP) = 16 V, collision energy (CE) = 25 eV and **27 eV** and collision exit potential (CXP) = 4 V and X V; ethinylestradiol **279 > 133** and 279 > 159, DP = 34 V, EP = 5 V, CEP = 15 V, CE = **25 eV** and 27 eV and CXP = 3 V; levonorgestrel 313> 109 and **313 > 245**, DP = 36 V, EP = 4.5 V, CEP = 18 V, CE = 29 eV and **21 eV** and CXP = 4 V and X V; drospirenone **367 > 97** and 367 > 197, DP = 41 V, EP = 6.5 V, CEP = 28 V, CE = **39 eV** and 31 eV and CXP = 4 V. The dwell time for each MRM transition was set 90 ms, and the collision cell was filled with the CID gas medium. The results were processed by Analyst software version 1.5.1 (Sciex, Canada).

RESULTS AND DISCUSSION

SPE Efficiency Comparisons. Prior to the SCB utilization as an SPE sorbent, the Oasis HLB cartridge was routinely employed for the study of synthetic hormones in the matrices specified in the Experimental Section procedure. To ascertain the SCB material's SPE efficacy, six replicates of the pool of industrial effluent sample spiked with 100 ng/mL of estradiol (analytical control) were extracted, and the comparative results among the cSPE materials are shown in Table 1. The SCB SPE results were significantly acceptable for all the compounds when compared to Oasis HLB−benzene copolymer SPE 30 μm 80A material (accuracy data ranged from 99% to 120%). Surprisingly, the carbon-based SPE material (Envi-Carb 100 mg) did not demonstrate recovery efficiency, despite its use as a suitable absorbent for removing or capturing hormones from various sample matrices.[27] The C_{18} cSPE bed demonstrated similar compatibility with previously published studies for this application.[28,29] Additionally, the repeatability (precision) results from the SCB SPE bed replicates ($n = 6$) were analytically and statistically satisfactory for the quantitative purpose shown in Table 2. It is critical to mention that the SPE load concentrations of each synthetic hormone in the industrial effluent sample were as follows: 0.024 μg of ethinylestradiol; 0.067 μg of levonorgestrel, and 0.65 μg of drospirenone, owing to the sample dilution factor of 100 and the load final volume of 1 mL.

Table 2. Reproducibility (Precision) Results from the Concentration Average of SCB 50 mg SPE of the Synthetic Hormones in Industrial Effluent

($n = 6$)	estradiol	ethinylestradiol	levonorgestrel	drospirenone
STD	11.9	0.245	0.608	0.408
CV (%)	8.65	10.1	9.03	0.628

SCB SPE Reusability (Recycling). cSPE cartridges are unambiguously disposable and recommended by vendors for a single procedure/application. The experiment demonstrated that SCB SPE cartridges can be reused up to three cycles with recoveries of <87% before exhibiting a decline in retention capacity. Table 3 presents the results of reusing SPE cartridges for three cycles as described in the Experimental Section. This study established the advantage of the SCB bed in terms of effectiveness and cost/benefit analysis.

Load SPE Concentration Variation (Load Capacity). To gain a better understanding of the absorption range and the linearity, we loaded the SCB 50 mg SPE cartridges with a range of synthetic hormone concentrations in water ranging from 0.05 to 2.0 μg/mL (estradiol was spiked at 100 ng/mL in all solutions as analytical control). This range was selected based on the solubility of the following compounds in water: ethinylestradiol (11.3 μg/mL), levonorgestrel (2.05 μg/mL), and drospirenone (1.81 μg/mL) (PubChem site). As illustrated in Table 4, the recoveries range between 80 and 108% for the minimum and maximum values, respectively. Estradiol recovered at an average rate of 90.2% in the same experiment, with a standard deviation (STD) of 5.26 and relative standard deviation (RSD) of 5.83 when six spiked replicate solutions were analyzed. Figure 1 demonstrates the linearity among the concentration level range. Considering the load sample volume of 1 mL in 50 mg of SCB bed, the quantity in mass of each synthetic hormone that passed through the SPE varied from 0.05 to 2.0 μg, and the total calculated capacity were from 1 to 40 ng/mg (total sum of the synthetic hormones from 3 to 120 ng/mg), respectively. These results

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Table 3. SCB50 mg SPE Cartridges Cycle Use Results in Concentration in Industrial Effluent Sample and Evaluation Recovery from Initial (1x)

	estradiol		ethinylestradiol		levonorgestrel		drospirenone		
SCB 50 mg SPE cartridges	concn (ηg/mL)	recovery (%)	concn (μg/mL)	recovery (%)	concn (μg/mL)	recovery (%)	concn (μg/mL)	recovery (%)	avg recovery (%)
1×	119	100	2.43	100	6.74	100	65.0	100	100
2×	102	85.7	2.05	84.5	5.58	82.8	62.0	95.4	87.1
3×	103	86.6	1.85	76.3	6.58	97.7	58.0	89.3	87.4
4×	69.9	58.7	1.23	50.7	4.62	68.6	49.0	75.4	63.4

Table 4. SCB 50 mg SPE Cartridges: Load Concentration (Capacity) in Water

	ethinylestradiol		levonorgestrel		drospirenone		
nominal concn (μg/mL)	concn (μg/mL)	recovery (%)	concn (μg/mL)	recovery (%)	concn (μg/mL)	recovery (%)	avg recovery (%)
0.05	0.047	94.2	0.054	108	0.0420	108	103
0.125	0.103	82.4	0.1332	107	0.1034	107	98.5
0.250	0.201	80.4	0.232	92.8	0.212	92.8	88.7
0.50	0.410	82.0	0.504	101	0.478	101	94.5
1.00	0.805	80.5	1.04	104	0.975	104	96.2
2.00	1.72	86.0	2.05	103	2.13	103	97.0

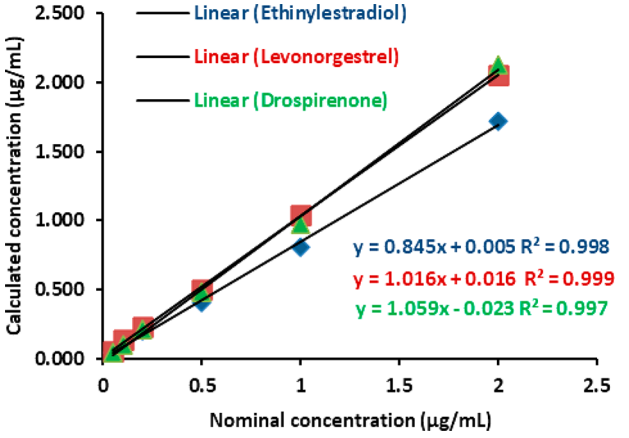

Figure 1. SCB 50 mg SPE linear capacity in load concentration in water.

demonstrate the importance of diluting the industrial effluent sample prior to the SPE load in order to maintain the retention capacity while reducing the undesirable matrix effect (interferences), breakthrough, and cartridge clogging (sorbent fouling).

SCB SPE Mass Variation. The purpose of this experiment was to determine the SCB retention capacity within the SCB bed filled mass. The industrial effluent samples were extracted under the same conditions described in the experimental section, except that the SCB bed mass contained was reduced to 10 mg and 25 mg. The results of the LC−MS/MS analysis were compared to the data obtained from the 50 mg SCB mass use. At 10 mg and 25 mg of SCB SPE filled mass, the results indicated an accuracy of 79.7% and 98.6%, respectively (on the compound average data). These results demonstrate that SCB bed material can be used in SPE up to a concentration of 25 mg without compromising retention capacity, thereby reducing material quantity while maintaining extraction efficiency.

SCB 50 mg SPE Breakthrough Evaluation. The breakthrough parameter defines the maximum volume of water sample which can be introduced into the SPE sorbent. This parameter depends on the kinetics of adsorption and desorption characteristics of the SPE bed, its hold-up volume, and the retention factor.[30] To evaluate the breakthrough of the SCB 50 mg bed, we proceed with a noncomplex assay, which consists of maintaining a fixed concentration of 1 μg/mL for each synthetic hormone in the SPE load aqueous solution and varying the load volume from 1; 10; 25, and 50 up to 100 mL. The eluted samples from different SPE volume experiments were quantified by LC−MS/MS. Figure 2 shows the recovery percentage results versus load volume.

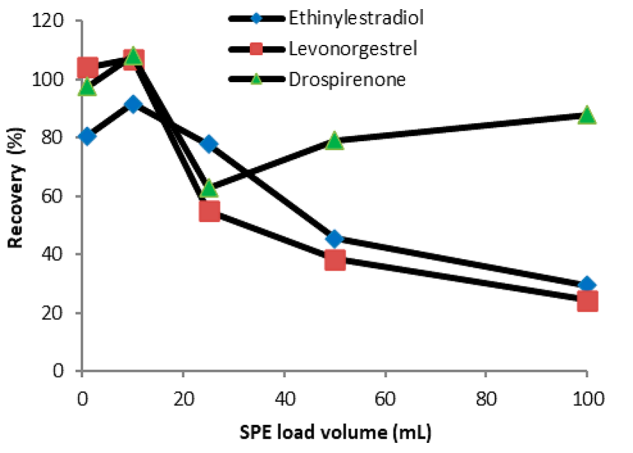

Figure 2. Relationship between the breakthrough volume and the SCB 50 mg SPE for the three synthetic hormones (1 μg/mL): ethinylestradiol, levonorgestrel, and drospirenone.

The results indicated that a volume capacity of 10 mL is the maximum or breakthrough volume without compromising synthetic hormone retention. The significant increase in recovery level at 50 mL load on drospirenone data is ascribed to a matrix effect in the analytical measurement, not to the effectiveness of the SPE. It represents a sum in the synthetic hormones mass load of 30 μg and considering 50 mg of SCB bed, resulting in a 0.6 μg/mg retention factor or experimental load capacity. This factor can facilitate in the scaling up of SPE procedures for the application of greater concentration levels of synthetic hormones or load volumes samples. Additionally, it can be extended to various absorptive compounds as a

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Table 5. SCB 50 mg SPE Cartridges: pH Difference on Retention Capacity (Fixed Concentration of Synthetic Hormones, 1 μg/mL)

load condition	ethinylestradiol recovery (%)	levonorgestrel recovery (%)	drospirenone recovery (%)	avg recovery (%)
10 mM HCl pH 1.2 (acid)	80.0	84.0	65.9	76.6
10 mM Na_2HPO_4 pH 7.4 (neutral)	62.8	72.7	52.9	62.8
10 mM NH_4HCO_3 pH 9.4 (basic)	85.9	95.8	74.2	85.3

Table 6. SCB 50 mg SPE Cartridges: Extraction Solvents Influence on Recovery Data (Fixed Concentration of Synthetic Hormones, 1 μg/mL)

extraction solvents	estradiol[a] recovery (%)	ethinylestradiol recovery (%)	levonorgestrel recovery (%)	drospirenone recovery (%)	avg recovery (%)
methanol	114	92.0	93.4	78.5	94.6
acetonitrile	102	99.8	99.5	80.4	95.5
isopropyl alcohol	104	85.7	87.7	56.0	83.4
ethanol	115	103	105	79.4	101
acetone	132	114	108	94.3	112
ethyl acetate	138	111	109	87.6	111
hexane	0.0	0.0	9.3	0.0	2.3

[a]Compound control at 100 ηg/mL.

starting point, demonstrating the versatility and applicability of the SCB 50 mg SPE.

pH Influence on the SCB Bed. To further understand the effect of pH on the SCB bed in the SPE experiment, the surface pH was measured using a 5% SCB w/w solution in water. The pH 7.26 value obtained suggests a neutral state when this solution is used in conjugation with the SPE developed method for extracting synthetic hormones from diluted industrial effluent samples that are likewise neutral in pH (7.20). To determine the effect of pH on the SCB 50 mg bed, three 1 mL load sample solutions were prepared at a fixed concentration of 1.0 μg/mL (each synthetic hormone): (a) 10 mM of HCl (pH 1.2), (b) 10 mM Na_2HPO_4 (pH 7.4), and (c) 10 mM NH_4HCO_3 (pH 9.5), and applied to the SPE procedure. The findings in Table 5 reveal lower recovery values (<80%) obtained in this experiment which indicated that the presence of electrolytes (cations and anions) can compete directly with the SCB binding sites, thus altering the kinetics of adsorption and desorption characteristics. Salker et al.[14] suggested some chemical interactions between organic compound and SCB material (ion exchange, chelation, and complexation). It can be a mixed mode of affinity and ion exchange, due to the presence of chemical lignocellulose biomass functional groups (acid and basic of Lewis). This explains the excellent adsorptive/affinity proprieties of certain organic compounds.

SCB 50 mg Using Different Extraction Solvents. The solvent used in the SPE procedure is paramount for establishing an optimal condition for obtaining recovery values between 80 and 120% (depending on the sample matrix and concentration) for selectively extracting the organic compounds from the sample. The main principle of SPE is to facilitate the separation of target analytes from complex sample matrices and keep their extraction free of interferences that impair the accuracy in the qualitative and quantitative measurements. The benefit of coupling SPE (on or offline) with LC−MS/MS (MRM mode) is that it provides a desired selectivity analysis, but the employment of other chromatographic techniques within lesser selective detectors such as UV−vis, FL, and others complicates SPE optimization. To gain a better understanding of the solvents that can be useful for SCB SPE application, a 1 μg/mL of aqueous solution of the three synthetic hormones (100 ng/mL estradiol) was loaded and extracted with 0.6 mL of each of the following solvents: methanol, acetonitrile, isopropyl alcohol, ethanol, acetone, ethyl acetate, and hexane. It is important to note that these solvents are often employed in SPE procedures, and it is preferred to utilize those that are less hazardous or toxic to humans or the environment. The recovery data in Table 6 demonstrate that all the solvents, except hexane, are acceptable for this application, with recovery values ranging between 83 and 112%. To assist in making a more informed selection about the solvent of choice for synthetic hormones, the following solvents are qualified: acetone > ethyl acetate > acetonitrile > ethanol > methanol > isopropyl alcohol > hexane. The study of cleanness, which is a prerequisite for cSPE bed certification, was conducted experimentally. The tested samples were injected into a mass spectrometer analyzer using Q3 in a trapping (1000 Da/sec) scan mode (150 a 1000 Da), maintaining the same chromatography and ionization settings throughout. The purpose of this test is to verify and rule out any potential interference with the quantity and intensity of ions. Isopropyl alcohol > ethanol > ethyl acetate > acetone > acetonitrile > methanol is the best solvent for SCB SPE bed under the described experimental conditions. These data demonstrate the versatility of various solvent systems for the SCB bed, corroborating the applicability of SCB SPE optimization, which depends on solvent availability/accessibility, material, and analytical instrumentation to deliver reliable, precise, and accurate quantitative results.

SCB Particle Size Bed Influence. This parameter evaluation is essential because the adsorption of synthetic hormones by the SCB bed can be influenced by particle size. In general, decreasing the particle bed size increases the contact surface area, thereby increasing adsorption capacity. To verify the SCB particle bed size adsorption capability we sieved the material with different diameter sizes: 20, 75, 125, 250, 355, 425, and 710 μm and 1 mm, collecting the SCB bed material in between the procedures, where we obtained the powder with average particle sizes of 75, 100, 187, 305, 390, and 567 μm. A 50 mg portion of the various sizes of SCB particle bed was loaded in the SPE cartridge, and the standard extracted procedure outlined in the Experimental Section was applied in the water solution sample at each synthetic hormone

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Table 7. SCB 50 mg SPE Cartridges: Particle Size Variation Evaluation (Fixed Concentration of Synthetic Hormones, 1 μg/mL)

SCB average particle bed size (μm)	estradiol[a] recovery (%)	ethinylestradiol recovery (%)	levonorgestrel recovery (%)	drospirenone recovery (%)	avg recovery (%)
75	93.3	99.5	99.5	88.0	95.1
100	90.7	104	104	88.5	96.6
187	84.3	91.8	91.8	81.8	87.4
303	62.9	78.6	78.6	79.0	74.8
390	59.2	71.4	71.4	85.7	71.9
567	42.2	54.1	54.1	70.6	55.2

[a]Compound control at 100 ηg/mL.

Table 8. Results from the Industrial Effluent Samples Using cSPE × SCB

	ethinylestradiol		drosperinone		levonorgestrel	
	HLB (30 mg)	SCB (50 mg)	HLB (30 mg)	SCB (50 mg)	HLB (30 mg)	SCB (50 mg)
effluent samples	concn mg/L	concn mg/L	concn mg/L	concn mg/L	concn mg/L	concn mg/L
Jul/2020	1.02	1.05	29.1	29.2	2.72	2.96
Aug/2020	0.691	1.01	3.81	3.20	0.878	1.02
Sep/2020	1.39	1.46	28.3	29.9	2.39	2.68
Oct/2020	1.56	1.95	5.94	6.63	3.39	3.39

concentration of 1.0 μg/mL and estradiol (control) at 100 ηg/mL. The results presented in Table 7 point out that 187 μm is the maximum limit SCB particle bed size for acceptable analytical usage (considering the established protocol) that reflects recoveries (average) above 85%.

Additionally, the data demonstrated the expected trend described above, where 75 μm provided the best recovery or adsorption results, while increasing the particle bed decreased recovery and adsorption. After the SPE experiment was completed, we examined the wastewater from the load solution and found a breakthrough effect as the particle size increased, for 75 μm, no compounds were detected, but at 567 μm, the average percentage (all the compounds) of the initial concentration was 20%. To ascertain if the findings achieved here were affected by the load solution contact time inside the bed, and since the SPE efficiency is also dependent on the applied flow rate (1.5 mL/min), we conducted SPE experiments offline. We utilized the 75 and 567 μm SCB bed material (in an Eppendorf 1.5 mL tube) for this experiment and followed the same SPE standard protocol, except that the contact time with the load solution was extended to 1 min through vortex agitation. Surprisingly, the recovery rates at 75 and 567 μm were comparable, as shown in the Supporting Information. The performed experiments demonstrated that the SBC bed has a high absorptivity or affinity for the examined hormones, and this information can be used to scale up SPE procedures or to remove them from effluents or wastewaters.

SCB SPE Applicability. We have utilized the established SPE protocol with the SCB bed to quantitatively measure synthetic hormones in industrial pharmaceutical effluent as part of the quality control monitoring program in industrial facilities. The sensitivity of the LC−APCI−MS/MS was measured through the estimated limit of detection (LOD; S/N = 3): estradiol, 1.0 ηg/mL; ethinylestradiol, 1.5 ηg/mL,; levonorgestrel, 0.69 ηg/mL; and drospirenone, 0.32 ηg/mL. The limit of quantification (LOQ; S/N = 10) is estradiol, 3.5 ηg/mL; ethinylestradiol, 5.0 ηg/mL; levonorgestrel, 2.3 ηg/mL; and drospirenone, 1.1 ηg/mL. The findings of the entrance effluent prior to the treatment procedure are shown in Table 8. As presented, the Oasis HLB (30 mg) bed results are comparable to those of the SCB (50 mg) bed and can therefore be used or replaced as a cost-effective alternative for extracting synthetic hormones and similar compounds from effluent/wastewater samples prior to qualitative and quantitative analysis.

CONCLUSIONS

Our overarching objective for developing sugar cane bagasse (SCB) as SPE sorbent material for the analysis of synthetic hormones from industrial effluents has expanded the research area and/or application of SCB, the biomass of which is often burnt to generate energy in the sugar and alcohol mills. Furthermore, our extensive investigations have established unequivocally that the SCB bed is an effective SPE sorbent material for the retention of synthetic hormones in industrial effluent samples, with the potential to expand to other hormones, steroids, and similar organic compounds and sample matrices, within the context of green chemistry. Scaling up SCB beds can be advantageous as a less expensive and more environmentally friendly method of removing hormones, steroids, and other emerging contaminants from wastewater and industrial effluents, thus contributing to environmental cleanup.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c06064.

Result table from particle size batch assay (SBC 75 and 567 μm) described in the end of results and discussion section; test from two different SCB bed preparation lots for SPE use (PDF)

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Notes

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