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# Thermally condensed humic acids onto silica as SPE for effective enrichment of glucocorticoids from environmental waters followed by HPLC-HESI-MS/MS

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## ABSTRACT

Pristine humic acids (HAs) were thermally condensed onto silica microparticles by a one-pot, inexpensive and green preparation route obtaining a mixed-mode sorbent (HA-C@silica) with good sorption affinity for glucocorticoids (GCs). The carbon-based material, characterized by various techniques, was indeed applied as the sorbent for fixed-bed solid-phase extraction of eight GCs from river water and wastewater treatment plant effluent, spiked at different concentration levels in the range 1–400 ng $L^{-1}$. After sample extraction, the target analytes were simultaneously and quantitatively eluted in a single fraction of methanol, achieving enrichment factor 4000 and 1000 in river water and wastewater effluent, respectively. Full recovery for all compounds, was gained in the real matrices studied (80–125% in river water, 79–126% in wastewater effluent), with inter-day precision showing relative standard deviations (RSD) below 15% and 18% ($n = 3$), for river and wastewater effluent, correspondingly. The high enrichment factors coupled with high-performance liquid chromatography tandem mass spectrometry quantification (MRM mode) provided method quantification limits of 0.009–0.48 ng $L^{-1}$ in river water and 0.06–3 ng $L^{-1}$ in wastewater effluent and, at the same time, secure identification of the selected drugs. As also evidenced by comparison with literature, HA-C@silica proved to be a valid alternative to the current commercial sorbents, in terms of extraction capability, enrichment factor, ease of preparation and cost. The batch-to-batch reproducibility was assessed by recovery tests on three independently prepared HA-C@silica powders (RSD lower than 7%).

## 1. Introduction

Belonging to the class of steroidal hormones, glucocorticoids (GCs) are drugs largely used in human medicine for a series of pathologies such as rheumatism, malignant tumours, skin diseases, Cushing syndrome etc., because of their ability to reduce inflammations and suppress allergic reactions and immune system response [1]. Anti-inflammatory and analgesic effects can inhibit muscle pain and raise the fatigue threshold; therefore GCs are presently banned by the World Anti-Doping Agency (WADA) during sporty competitions [2]. The same pharmacological properties stand for the wide administration of GCs as feed additives to animals in livestock farming, although since 1990 in European Union they are forbidden for use as growth promoters [3].

The widespread use of these drugs in our daily life unavoidably leads to a continuous release in the environment, mainly due to poor metabolization (50–90% of the ingested dose is excreted via urine and faeces with no structural modifications) [4], direct release from ointments/creams, and, ultimately, because of the partial abatement of wastewater treatment plants (WWTPs) [4,5]. Direct addition in water for aquaculture practices should also be taken into account as a further contamination route [4].

The environmental diffusion of these xenobiotic compounds would bring potential adverse effects towards animals, e.g. inhibition of locomotion, aggressive behaviour and possible modification

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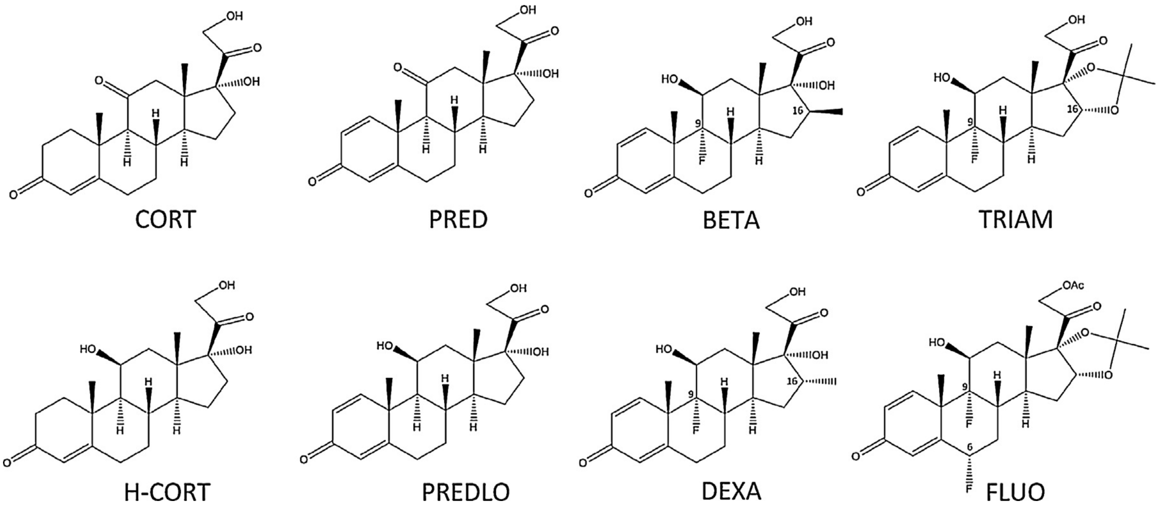

**Fig. 1.** Molecular structures of the target analytes.

of the sexual life [6–8], and also to humans due to bioaccumulation in the food chain as a consequence of their lipophilicity [9,10]. In this context, the EU regulations have established maximum residue limits to ban illicit administration of some kinds of GCs in animal feed; in particular limits of 0.3 $\mu$g kg$^{-1}$ and 0.6 $\mu$g kg$^{-1}$ were set for betamethasone/dexamethasone and prednisolone, respectively [9]. On the other hand, it should be noticed that no trigger values have been established at present for environmental waters. The actual concentration levels in these matrices are quite variable, ranging from few to some hundred nanograms per litre [4,11–15], with higher values locally determined in hospital wastewater (in the range 0.3–2 $\mu$g L$^{-1}$ [16]).

The current analytical methods for determination of GCs in aqueous matrices essentially rely on liquid chromatography-tandem mass spectrometry (LC–MS), which provides a rapid and accurate detection and it is highly preferred to gas chromatography that instead requires tedious derivatization steps [9]. However, the low concentrations of GCs commonly present in environmental waters require a pre-concentration step before LC–MS, mainly performed by solid-phase extraction (SPE). Most of the sorbents currently used are commercial, polymeric phases such as HLB (*N*-vinylpyrrolidone-divinylbenzene copolymer), Strata-X (*N*-vinylpyrrolidone chemically modified divinylbenzene polymer) and hydrophilic divinylbenzene, able to retain GCs both by hydrophobic and hydrophilic interplays [11–13,15,17,18]. These sorbents are well known mixed-mode materials, similarly to graphitic carbon nitride [19,20] and graphene [21,22], the latter used also for extraction/cleanup of GCs [9,23].

On the basis of the present state of the art, aim and novelty of this work are the green preparation of a new carbon-based SPE sorbent to be used for determination of eight GCs in natural waters, *viz.* cortisone (CORT), hydrocortisone (H-CORT), prednisone (PRED), prednisolone (PREDLO), betamethasone (BETA), dexamethasone (DEXA), triamcinolone acetonide (TRIAM) and fluocinolone acetonide acetate (FLUO). In order to have a cost-effective material applicable in conventional column-SPE, humic acids (HAs) were immobilized onto micrometric silica by anoxic thermal treatment, a successful approach for immobilization of graphene-like carbons onto silica [24,25].

With regard to HAs, the proposed chemical structure has an aromatic ring of di- or tri- hydroxyphenol type bridged by —O—, —CH—, —NH—, —N=, and other groups such as carboxylic and phenolic functionalities both contributing most to surface charge and reactivity of HAs [26]. For this reason, Erny et al. [27] investigated the potentiality of immobilized humic substances for SPE of triazines.

In the present study, HAs were selected as the *carbon source* in view of their low cost and, foremost, because their macromolecular base structure, consisting of hydrophobic frameworks and abundant hydrophilic groups [26], seems prone to be converted into an aromatic carbonaceous phase containing oxygenated functionalities, by a simple pyrolytic treatment. This, directly performed on the silica + HAs dried mixture (previously homogenized in water suspension), yields a new *mixed-mode* carbonaceous phase supported on the inorganic particles, prepared avoiding use of organic solvents and time-consuming multi-step reactions. Most importantly, the designed sorbent (HA-C@silica) is expected to have good sorption affinity for GCs (and other compounds of similar structure), made of an apolar nucleus bearing polar oxygenated groups (Fig. 1), due to solute-sorbent multi-type interactions, e.g. $\pi$ stacking, dipole-dipole and H-bond.

HA-C@silica, characterized by scanning electron microscopy (SEM), surface area measurements (BET method), thermogravimetric analysis (TGA) and X-ray photoelectron spectroscopy (XPS), was tested for the pre-concentration of eight widely used GCs from tap, river and urban wastewater effluent samples, prior to high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC-HESI-MS/MS), multiple reaction monitoring (MRM). The analytical method was applied to the determination of the target molecules in natural-contaminated water samples. The overall performance obtained in terms of recovery, enrichment factor (EF), sensitivity, and cost, was discussed in comparison with the analytical methods currently available in the literature, based on commercial SPE sorbents.

## 2. Experimental

### 2.1. Chemicals and materials

All chemicals were reagent grade or higher in quality. Silica microparticles (40–63 $\mu$m, surface area 550 m$^2$ g$^{-1}$, pore volume 0.8 cm$^3$ g$^{-1}$), humic acids sodium salt (technical grade), high purity GCs standards (CORT, H-CORT, PRED, PREDLO, BETA, DEXA), acetic acid (AA, 99–100%), polypropylene tubes and polyethylene frits were supplied by Sigma-Aldrich (Milan, Italy). Analytical grade TRIAM and FLUO standards were purchased from Farmabios (Groppello Cairoli, Italy). HPLC gradient grade methanol (MeOH) and ultrapure water were supplied by VWR (Milan, Italy).

GCs stock solutions of 100 mg L$^{-1}$ were prepared in MeOH and stored in the dark (4 °C). GCs working solutions of 5–500 $\mu$g L$^{-1}$