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Fluoroquinolone residues in compost by green enhanced microwave-assisted extraction followed by ultra performance liquid chromatography tandem mass spectrometry

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ABSTRACT

A novel, simple and straightforward method for determination of fluoroquinolones (FQs) in compost has been developed. The procedure entails a low-pressurized microwave-assisted extraction (MAE) carried out by a high performance instrument, in alkaline aqueous solution containing magnesium ions as FQs complexing agent, followed by ultra performance liquid chromatography tandem mass spectrometry (UPLC–MS). Ciprofloxacin (CIP), Enrofloxacin (ENR), Levofloxacin (LEV) and Norfloxacin (NOR), four widely used FQ antibiotics, were simultaneously extracted from compost by a *single* MAE cycle (20 min, 135 °C). The method was validated in terms of linearity, selectivity, sensitivity and accuracy. Quantitative absolute recovery (70–112%, $n = 3$) and suitable precision (RSD < 15%, $n = 3$) were observed, at concentration levels ranging from 25 ng g^{-1} to 2500 ng g^{-1}. Analytes were separated in a 10 min chromatographic run and quantified/confirmed in single reaction monitoring (SRM) mode. UPLC coupled to SRM-MS detection allowed to achieve improved sensitivity, and selective detection. Method detection and quantification limits, MDLs and MQLs, were in the range 2.2–3.0 ng g^{-1} and 6.6–9.0 ng g^{-1}, respectively. The high-performance microwave system here used strongly improved the extraction efficiency with respect to a conventional apparatus. The procedure proved to be simpler, less expensive, faster, and more green with respect to the few methods currently described in literature, providing at the same time suitable recovery and reproducibility. The analytical method has been applied to the analysis of actual compost samples, wherein FQs have been quantified at concentrations up to 88 ng g^{-1}.

1. Introduction

In the last two decades the amount of sewage sludge has increased dramatically due to the growth in urban population and thus livestock farming [1]. Besides being regarded as an undesired waste, sewage sludge can be conveniently recycled for agricultural purposes as a low-cost organic biomass. Composting or aerobic biological treatment of organic wastes is a common practice to reduce wastes and to exploit organic matter and inorganic nutrients (e.g. phosphorous, nitrogen); in particular, composting is an economical and sustainable approach for converting sewage sludge into a final product with a high organic content that is largely and conveniently used as soil conditioner and/or fertilizer [1]. On the other side, direct field application of raw sludge is not convenient because of leaching of nutrients and the environmental pollution that would derive from the widespread of untreated sludge [2]. With regard to this, several pharmaceuticals have been determined in sewage sludge at micrograms *per* kilogram levels [3], among which residuals of antibiotics such as fluoroquinolones (FQs), sulfonamides and tetracyclines [4]. It has been demonstrated that, despite biodegradation possibly taking place during the composting process [5], variable amounts of residual drugs can be found also in the final product. In particular, a recent paper [2] reported that Ciprofloxacin (CIP), one of the most widely prescribed FQ in the world [6], shows higher persistence during composting than other pharmaceuticals such as sulfadiazine and chlortetracycline, as a further evidence for FQs resistance to biodegradation [6]. Despite this poses the question of environmental diffusion of pharmaceutically active compounds, the presence of FQs antibiotics in compost has received little attention.

FQs are one of the most commonly employed class of antibacterial agents, adopted both for human and veterinary medicine. The

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target proteins of FQ drugs are bacterial DNA gyrase and topoisomerase IV enzymes, essential for DNA replication and transcription. In particular, the fluorine atom at C-6 position of the ring provides a more than 10-fold increase in gyrase inhibition and up to 100-fold improvement in minimum inhibitory concentrations, while substituent groups at position C-7 play a key role in determining the antibacterial spectrum and bioavailability; piperazine is frequently used and grants potency against Gram-negative bacteria. Due to their peculiar pharmacological properties, above all good oral intake and broad activity spectrum, they gained worldwide popularity [7].

At the same time, FQs have been included in the list of the "emerging pollutants", defined as new chemicals that have no regulatory status but may have an adverse impact on the environment and human health [8]. The environmental diffusion of these synthetic drugs has been assessed in the recent years, both in water [9,10] and soil [11] compartments, and essentially is involved by the partial metabolism FQs undergo after ingestion [12], to the partial removal during wastewater treatment [13,14] and land application of livestock/urban compost [15]. As a matter of fact, FQs are one of the most frequently detected pharmaceuticals, together with sulfonamides, tetracycline, and macrolides [12].

Although photochemical degradation can alleviate their accumulation in natural ecosystems [16–19], resistance to biodegradation and strong adsorption on solid matrices are responsible for their enhanced persistence [20]. From the environmental viewpoint, FQs diffusion is reason of great concern due to their capability to induce bacterial resistance [21–23], genotoxicity [24] and ecotoxicity [25–27]; moreover, their overall environmental impact is also due to the formation of various photoproducts able to exert themselves antibiotic activity [12,28]. For these reasons the development of novel treatments for environmental remediation is of overwhelming importance [29–31].

Presently, no trigger values exist for FQs in environmental matrices, although the general concentration of drugs should not exceed 0.1 $\mu g\ L^{-1}$ in groundwater, 100 $\mu g\ kg^{-1}$ in manure and 10 $\mu g\ kg^{-1}$ in soil [32]. Notice that CIP has been determined at far higher concentrations in manure, up to ca. 30 mg kg^{-1} [2], and it has been demonstrated that up to 30% of the CIP initial amount remains in the composting mass after manure storage [2].

Although only very few data are available in the literature, FQs have been detected in compost at the nanograms *per* gram levels [1,4,33]. It seems evident that using compost in agriculture represents an important route for FQs environmental pollution. This highlights the need for analytical tools suitable for monitoring the levels of such pharmaceuticals in this very complex, from the analytical point of view, humus-like matrix, and more generally to deeper assess the fate of these pharmaceuticals alongside sludge treatment [3].

The main challenge in trace-level determination in compost is to find out working strategies to minimize matrix interferences. This means improving selectivity, both in sample preparation and in detection, and sensitivity towards the target compounds.

For FQs determination in compost samples, Lillenberg et al. [1] applied the analytical method initially designed and validated on sewage sludge [4]. The procedure entailed 5 consecutive pressurized liquid extraction (PLE) cycles followed by solid-phase extraction (SPE) cleanup prior liquid-chromatography tandem mass spectrometry (LC–MS). A modification of the USEPA method 1694 was applied by Selvam et al. [2] to extract CIP from compost, performing a 3-cycles ultrasonic-assisted extraction (UAE) followed by SPE and evaporation of the SPE extract prior LC–MS. A single MAE cycle at drastic conditions using acetonitrile and 1% phosphoric acid, followed by salt-assisted liquid–liquid extraction cleanup and dispersive SPE, has been recently developed for quantitative recovery of quinolone antibiotics [33].

To the authors' best knowledge, no other analytical method is currently available in the literature for the extraction and determination of FQs in compost.

On the basis of this background, we developed a straightforward and green analytical method for simultaneous extraction of four widely used FQs – CIP, Enrofloxacin (ENR), Levofloxacin (LEV) and NOR – in compost, at the nano/micro-grams *per* gram concentration levels, based on a single MAE cycle, followed by ultra performance liquid chromatography electrospray ionization tandem mass spectrometry (UPLC–ESI-MS). The use of a new low-pressurized microwave platform allowed mild and highly efficient extraction of the analytes from compost, followed by SPE, and UPLC–MS. Notably, conventional MAE, already used for the extraction of FQs from soil, sludge and other solid matrices, did not provide suitable recoveries, due to the complexity of this matrix. The analytical figures of merit of the method, i.e. linearity, selectivity, sensitivity, and accuracy (trueness and precision) have been explicated and the final procedure has been applied to the analysis of commercial compost samples.

2. Experimental

2.1. Chemicals and materials

All the chemicals employed were reagent grade or higher in quality and used with no further purification. All FQs (CIP, ENR, LEV, NOR), HCOOH (≥96%), methanol (≥99.9%), UPLC–MS grade methanol, UPLC–MS grade HCOOH and hexahydrate $Mg(NO_3)_2$ (97%) were supplied by Sigma–Aldrich (Milan, Italy). HPLC gradient grade acetonitrile (ACN) was purchased by VWR (Milan, Italy). Ultra-pure water (resistivity 18.2 $M\Omega\ cm^{-1}$ at 25 °C) was produced in laboratory by a Millipore Milli-Q system. Anhydrous NaOH pellets (97%), H_3PO_4 (85%, w/w) and NH_3 (30%, v/v) were obtained from Carlo Erba Reagents (Milan, Italy). Oasis® HLB (60 mg) cartridges were purchased from Waters (Milan, Italy). FQs stock solutions of 300 $\mu g\ mL^{-1}$ were prepared in methanol containing 0.1% (v/v) 1 M NaOH, and stored in the dark at 4 °C for a maximum of three months. FQs working solutions of 0.04–4 $\mu g\ mL^{-1}$ in methanol were renewed daily. All the laboratory operations involving use of standard solutions were conducted under red light.

2.2. Instruments and apparatus

A sequential low-pressurized microwave solvent extraction system, equipped with a volume independent IR temperature sensor, electromagnetic stirring and cooling device (Discover SP, CEM S.r.l., Cologno al Serio, Italy) was employed. A Sigma 2-16P centrifuge (Celbio S.p.a., Pero, Italy) was used after sample extraction.

The chromatographic analysis was performed with a JASCO (Lecco, Italy) X-LC system interfaced with a Thermo Scientific (Milan, Italy) LTQ XL HESI-MS/MS system. An Agilent EC-C18 Poroshell column (2.1 mm × 50 mm, 2.7 μm) equipped with a similar pre-column was used.

2.3. Sample collection and storage

Commercialized samples of compost (10 kg) were purchased from a composting plant located in northern Italy, and their physical-chemical parameters can be found in Table S1 (Supplementary Data). Samples were left to dry at room temperature, homogenized, sieved (0.2 mm) and stored in the dark at 4 °C until analysis. Blank compost sample previously analyzed for the native FQs content [4] was used for the method development. Aliquots (0.3 g) of the as prepared samples were fortified at different concentration levels into 5 mL weight-boats and stored in the dark