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Novel cation selective exhaustive injection-sweeping procedure for 5-nitroimidazole determination in waters by micellar electrokinetic chromatography using dispersive liquid–liquid microextraction☆

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ABSTRACT

A novel method consisting of cation-selective exhaustive injection and sweeping (CSEI-sweeping) as on-line preconcentration followed by a micellar electrokinetic chromatography (MEKC) separation has been developed for the determination of 5-nitroimidazoles (5-NDZ) in environmental waters. Moreover, dispersive liquid–liquid microextraction (DLLME) has been proposed for first time as sample treatment technique prior to CSEI-sweeping-MEKC. DLLME was applied to 5 mL of sample. Dibromomethane (1156 μL) and 2-butanol (1363 μL) were employed as extractant and dispersive solvents, respectively. Salting-out effect was achieved by the addition of 16% (w/v) NaCl to the samples. After DLLME and organic solvent evaporation, the residue was redissolved in a low conductivity solvent (5 mM phosphoric acid with 5% of methanol) and electrokinetically injected at 9.8 kV for 632 s in a bare fused-silica capillary (57.2 cm, 50 μm I.D.). Prior to the injection, the capillary was rinsed with 50 mM phosphate buffer pH 2.5, followed by a plug of a higher conductivity buffer (100 mM phosphate pH 2.5, 50 mbar, 264 s) and a plug of water (50 mbar, 2 s). Separation was carried out applying −30 kV at 20 °C in 44 mM phosphate buffer pH 2.5, containing 8% tetrahydrofuran and 123 mM sodium dodecyl sulfate. Analytical signals were monitored at 276 nm. Validation was performed in river and well waters, obtaining satisfactory results in terms of linearity, precision (% RSD generally lower than 10%) and trueness (recoveries higher than 70% in almost all cases). LODs ranged from 0.61 to 2.44 ng/mL. The combination of this microextraction technique with the proposed capillary electrophoresis methodology supposes a simple, sensitive and cheap alternative for 5-NDZ analyses, in accordance with the aims of green chemistry.

1. Introduction

Metronidazole (MNZ) is an antibiotic which belongs to 5-nitroimidazole (5-NDZ) family. It is widely used in humans for treating diseases due to anaerobia microbes [1]. It is considered an essential antibiotic according to World Health Organization (WHO) [2]. Other drugs from the same family, such as tinidazole (TNZ), ornidazole (ORZ), carnidazole (CRZ), ipronidazole (IPZ), secnidazole (SCZ) and ternidazole (TRZ) have been also considered in the current work. The chemical structures of the selected compounds are shown in Fig. 1. In spite of the effectiveness of 5-NDZs as antibacterial and antiprotozoal agents, their employment in the veterinary practice is restricted. Some reports attribute mutagenic, carcinogenic and genotoxic properties to 5-NDZs [3,4], and therefore their application have been banned in animals intended for human consumption in European Union (EU), United States (US) and China [5–7]. On the other hand, 5-NDZs possess high polarity and low biodegradability, which involves high bioaccumulation levels and, consequently, ecotoxicity [8]. The presence of antibiotics such as 5-NDZs in sewage water has been already reported [9], even though a few number of papers have been focused on their removal from wastewater treatment plants [10,11]. For all these reasons, 5-NDZ drugs are considered as emerging water micropollutants. The environmental risks caused by drugs have been studied in the past decade [12], however more research in this area is still required, including updated reports about their presence and levels in ecosystems. Thus, analytical methodology for detection

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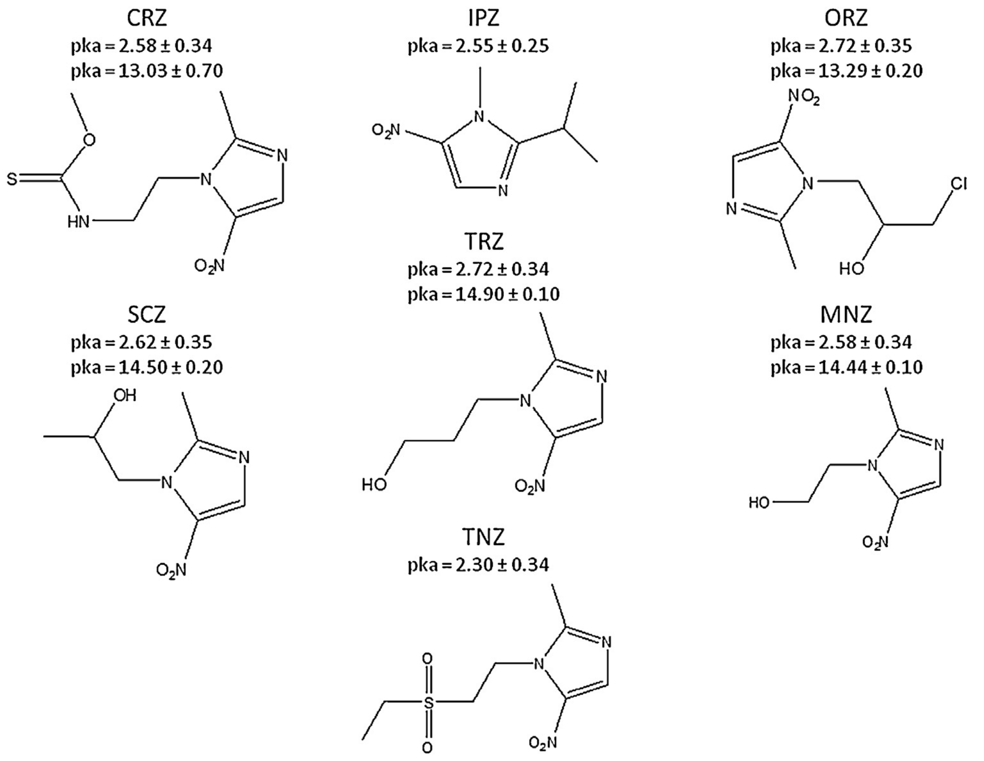

Fig. 1. Chemical structures of evaluated 5-NDZ compounds. *p*K*a values have been obtained from Scifinder® database.

and quantification of these residues in the environment is highly needed in order to evaluate the exposition of the environment to antibiotics and their risks [13].

Several methods have been proposed for 5-NDZ determination [14–17], however, applications to environmental water samples are reduced [18,19,20]. New contributions about 5-NDZ determination in aquatic environmental samples are desired, taking into account that their presence has been already reported in natural waters [21]. Traditionally, liquid chromatography (LC) coupled to mass spectrometry (MS) has been the most popular choice for monitoring 5-NDZ residues [22,23]. Capillary electrophoresis (CE) [24] and gas chromatography (GC) [25] are among the proposed alternatives to LC. Low solvent consumption, short analysis time and high efficiency are CE characteristics; however its use is limited due mainly to the poor sensitivity, especially when it is coupled to ultraviolet (UV) detection. In order to overcome this disadvantage, different preconcentration strategies have been developed, including on-line (sample stacking techniques) [26], in-line [27] and off-line (through sample pretreatment) procedures [28]. The most common on-line preconcentration methodologies include: acetonitrile stacking [29], field-amplified sample stacking (FASS) and field-amplified sample injection (FASI) [30], sweeping [31], dynamic pH junction [32] and isotachophoretic stacking [33], although others less known strategies offer even higher sensitivity enhancement factors (SEF).

A decade ago, Quirino et al. proposed a novel on-line preconcentracion technique based on cation-selective exhaustive injection and sweeping (CSEI-sweeping). It combines two on-line preconcentration techniques: field-enhanced sample injection (FESI) and sweeping. This combination allows achieving enhancement factors from a thousand- to almost a million-fold in relation to conventional CE [34]. CSEI-sweeping involves electrokinetic injection (FESI) of a high amount of charged cationic compounds, creating long analyte zones in the capillary with higher concentration than in the original sample solution [35]. After sample injection, background electrolyte (BGE) vials containing micelles are placed at both ends of the capillary and negative voltage is applied. Micelles are focusing the analytes in narrow bands by sweeping, and they are consequently separated by conventional micellar electrokinetic chromatography (MEKC). A procedure scheme is shown in Fig. 2. To achieve FESI, analytes must be dissolved in a low-conductivity medium, which is not so obvious, especially for complex real samples showing high or moderate salinity. In such cases, a proper sample pretreatment is needed in order to reduce sample

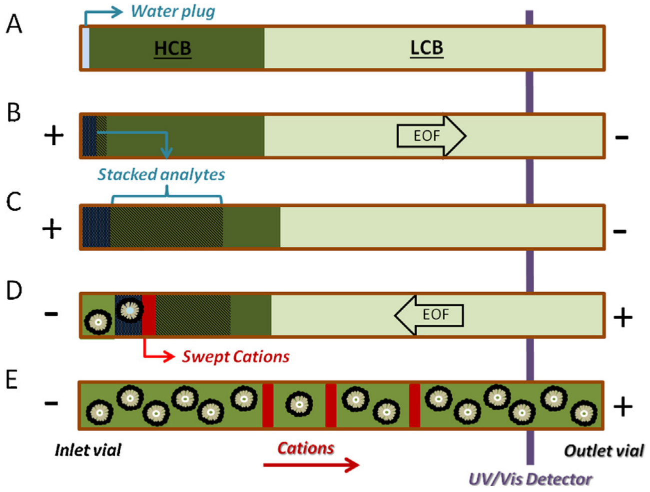

Fig. 2. CSEI-Sweeping-MEKC procedure scheme. Adapted from reference [34]. Steps: A, capillary is rinsed with a low conductivity buffer (LCB), followed by a plug of a higher conductivity buffer (HCB) and a water plug; B, electrokinetic injection at positive polarity, being cationic analytes stacked at the interface between the water zone and the HCB zone; C, cationic analytes are stacked at the HCB zone because of the long injection, but not at the water or matrix zone; D, background electrolyte is placed in both ends of the capillary and a negative voltage is applied; E, ordinary MEKC separation takes place.