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Determination of 5-nitroimidazole residues in milk by capillary electrochromatography with packed C_{18} silica beds

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ABSTRACT

This work presents a novel methodology for analysing 5-nitroimidazole residues in milk samples by capillary electrochromatography using lab-made packed columns, produced by carrying out a high pressure packing procedure using acetone as driving solvent and C_{18} silica uncapped particles (5 μm particle size) as packing material. Column frits resulted from sintering the proper stationary phase by heating the packed material for 20 s with a nichrome ribbon (80% Ni–20% Cr, 28 cm × 2 mm × 0.2 mm, electric resistance 1.3 Ω) connected to a 7 V AC power supply. Lab-made C_{18} silica packed capillaries (40 cmx50 μm i.d.) were employed for the determination of 5-nitroimidazole drugs. Milk samples were treated by a salting-out assisted liquid–liquid extraction followed by a solid phase extraction with Oasis®HLB cartridges prior to their injection. Samples were hydrodynamically injected into the column for 120 s at 11.5 bar. Afterwards eight 5-nitroimidazole compounds were separated in isocratic mode under an applied voltage of 27 kV and a temperature of 30 °C. The selected mobile phase consisted of a mixture 60:40 acetonitrile:ammonium acetate (2.5 mM, pH=5). Separation was monitored at 320 nm and it was performed in less than 15 min. The method was characterized in terms of linearity ($R^2 \geq 0.993$) and precision (repeatability, RSD ≤ 12.2% and reproducibility, RSD ≤ 14.5%), obtaining detection limits lower than 29 μg/L for all compounds under study.

1. Introduction

5-Nitroimidazoles (5-NDZs) are constituted by an imidazole ring and a nitro group in fifth position. These antibiotics are used in human medicine against most Gram negative and some Gram positive anaerobic bacteria as well as against anaerobic protozoans. However, resistance bacteria against 5-NDZ based compounds have been reported [1] and other studies have attributed carcinogenic, genotoxic and mutagenic properties to 5-NDZ drugs [2,3]. In this sense, considering that residues of these substances could represent a risk to human health, their use in veterinary medicine is restricted, being banned in food-producing animals. European Union (EU) regulation [4] sets that 5-NDZ residues cannot be found in animal products intended to human consumption, but there are still alerts collected by the Rapid Alert System for Food and Feed (RASFF) about the presence of these compounds in some foods derived from animals [5]. Furthermore, the use of 5-NDZ antibiotics in veterinary medicine has also been forbidden in other countries such as China [6] and the United States of America (USA) [7]. Considering the globalization of food production, any alert about the illegal use of these antibiotics should be taken into account as international food safety concern. Therefore, simple, cheap and green analytical methodologies are required in order to detect 5-NDZ residues in animal derived foodstuffs.

During the last years several contributions have been reported about analytical methods intended to determine 5-NDZ in food. Liquid chromatography (LC) with UV [8–10] or mass spectrometry (MS) [11–14] detection is the most employed technique. Also, gas chromatography (GC) coupled to MS [15,16] or capillary electrophoresis (CE) with UV detection [17,18] have been proposed. The high solvent consumption and waste generation is the main drawback of LC methods, which could be avoided by employing greener techniques such as CE. However, the applicability of CE is limited due to the low sensitivity reached by this methodology as consequence of the injection of low sample volumes. In order to combine the advantages of both methodologies, capillary electrochromatography (CEC) has been proposed as a hybrid separation technique which shows high efficiency and selectivity [19].

In a CEC separation, the mobile phase is propelled through the stationary phase into the capillary by the electroosmotic flow (EOF) generated as a result of applying an electric field instead of a

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hydraulic pressure. The plug-like profile of EOF involves much higher CEC column efficiency than that reached by a pressure-induced flow at the same linear velocity in the same column, resulting in a peak resolution improvement [20]. In addition, CEC presents a dual separation mechanism based on differences among analyte electrophoretic mobility and analyte–stationary phase interactions. This combined mechanism gives a unique selectivity to CEC methods [21]. Three different CEC modalities can be considered depending on the stationary phase morphology: open tubular, monolithic and packed CEC. Open tubular capillaries offer poor sensitivity as consequence of the narrow bore size of the capillary and low separation capability due to the low phase ratio caused by the limited surface area of the stationary phase [22]. On the other hand, monolithic capillaries can suffer from a lack of stability because of polymeric monolithic stationary phases tend to swell in organic solvents. Moreover, the preparation of polymeric monoliths usually leads to micropores which results in low efficiency and peak asymmetry. Monolithic capillaries also possess low column capacity attributed to their low specific surface area, providing some limited applications [23]. In contrast to the above mentioned types of columns, packed capillaries offer higher surface area which improves sample loading capacity. The success of these packed capillaries lies in this advantage, becoming the most commonly employed CEC capillaries. They are commercially available but the problems of bubble formation, column fragility and above all, their high price, retard their extensive use [24] so, researchers often prepare their own columns and as a result, several protocols have been proposed for packing capillaries. However, some authors still consider these procedures too arduous, an art or even as "black magic" [25] methodologies because they require specific skills to achieve highly efficient and reproducible capillaries.

Since packed columns in CEC were introduced by Pretorius et al. in 1974 [26], different packing techniques have been tested in order to obtain the perfect packed column, even comparisons among them have been reported [27–29]. But in the last years, all of them have a common challenge that is to avoid poorly packed capillaries which can lead to low efficiency, poor resolution and asymmetric peaks. Several procedures for capillary packing have been described [25] including electrokinetic packing [30,31], packing with supercritical carbon dioxide [32], with centripetal forces [33] or by gravity [34]. However slurry packing with pressure [35–37] is the most established methodology because it is simple to implement and it does not require long recipes for start-up. The main difficulty of column packing resides in the production of the frits because they have to retain the stationary phase in the capillary when a separation voltage is applied and, at the same time, to allow the mobile phase to penetrate freely through them. If frits are too thin, they will not retain the sorbent, and if they are too thick and non-porous, the mobile phase will not pass through them. In these packed columns, EOF is not homogeneous along the capillary, showing different zones (packed part, frit and open part) which produce pressure differences across the frit [38]. As a result, bubbles are formed in the boundary region between the frit and the unpacked part of the capillary, causing lost of efficiency and current disruptions. In order to solve these problems, the use of different kind of frits, such as monolithic [39], sol–gel [40], magnetic [41], single particle [42] as well as new methods for sintering silica particles [43] have been proposed and compared [44,45]. Moreover, methodologies that attempt to carry out CEC separations in fritless capillaries have been recently reported [46,47]. Therefore, the major challenge of the packing procedure is the satisfactory fabrication of retention frits. Excluding methodologies such as single particle and internal taper approaches, sintering is the prevailing mode in frit fabrication, because it leads to the least frit-related band broadening [24].

The aim of this work is to propose an easy methodology for the fabrication of packed capillaries and their subsequent use for the monitoring of 5-NDZ residues by CEC. At the best of our knowledge, there is just one CEC method published for the determination of 5-NDZs [48]. Nevertheless, this work is focused on monolithic MIP-based capillary development and 5-NDZ standard separation is proposed without any application to real matrixes. In this work, 5-NDZ drugs are analysed for the first time by CEC using packed capillaries and the proposed methodology is evaluated for the analyses of milk samples. It is worth to mention that most of the papers about CEC are focused on testing new sorbents or developing new procedures to make CEC columns. However applications of these developments to solve practical problems with real samples are not usually carried out. In this sense, CEC remains as a technique with a great potential to be explored and this work could contribute to establish CEC as a mature technique that can move from research to routine laboratories.

2. Experimental

2.1. Materials and reagents

All reagents were analytical reagent grade, unless indicated otherwise, and solvents were HPLC grade. Ammonia (30%), sulphuric acid (98%), sodium chloride (NaCl) and sodium hydroxide (NaOH) were obtained from Panreac-Química (Madrid, Spain). Methanol (MeOH) and acetone were purchased from VWR International (West Chester, PA, USA) while acetonitrile (MeCN) and acetic acid were supplied by Sigma-Aldrich (St. Louis, MO, USA). Formic acid (98–100%), tris(hydroxymethyl)aminomethane (TRIS) and hydrochloric acid (HCl) 37% were acquired from Merck (Darmstadt, Germany). Ultrapure water (Milli-Q plus system, Millipore, Bedford, MA, USA) was used throughout the work.

Analytical standards of dimetridazole (DMZ; 1,2-dimethyl-5-nitroimidazole), ronidazole (RNZ; 1-methyl-2-(carbamoylmethyl)-5-nitroimidazole), carnidazole (CRZ; [2-(2-methyl-5-nitro-imidazol-1-yl) ethyl]thiocarbamic acid o-methyl ester), ornidazole (ORZ; 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole), metronidazole (MNZ; 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) and tinidazole (TNZ; 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole) were supplied by Sigma-Aldrich (St. Louis, MO, USA) while ipronidazole (IPZ; 2-isopropyl-1-methyl-5-nitroimidazole), secnidazole (SCZ; α,2-dimethyl-5-nitro-1 H-imidazole-1-ethanol hemihydrate) and ternidazole (TRZ; 1-(3-hydroxypropyl)-2-methyl-5-nitroimidazole) hydrochloride were purchased from Witega (Berlin, Germany).

Packed columns consisted of uncoated fused silica capillaries of 50 μm, 75 μm and 100 μm internal diameter (i.d.) which were purchased from Polymicro Technologies (Phoenix, AZ, USA) and LiChrospher RP-C18 non-encapped particles (5 μm particle size) (Agilent Technologies, Waldbronn, Germany) which were recycled from a damage LC column.

Oasis®HLB cartridges (60 mg, 3 cc) (Waters, Milford, MA, USA) were considered for the sample treatment procedure. Clearinert™ 13 mm syringe filters with 0.22 μm nylon membrane (Wilmington, DE, USA) were used for sample filtration prior to sample injection into the CEC system.

2.2. Standard preparation

Individual standard solutions of each 5-NDZ were prepared at 1000 mg/L by dissolving each pure compound in MeCN. These solutions were stored in dark bottles at −20 °C and equilibrated to room temperature before use. They were stable for at least six months.

Intermediate standard solution containing 100 mg/L of each