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Electric field-assisted solid phase extraction and cleanup of ionic compounds in complex food matrices: Fluoroquinolones in eggs

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ABSTRACT

The use of electric fields as additional driving forces in sample preparation techniques is an innovative approach that is environmentally friendly, straightforward, and able to overcome several limitations of conventional sample preparation procedures. In this work, the advantages of electric field-assisted solid phase extraction (E-SPE) using syringe-type cartridges were demonstrated for the extraction of four fluoroquinolones (FQs) in their anionic forms. The FQs were extracted from eggs and subsequently determined by UHPLC–MS/MS. The use of electric fields during the washing and final elution steps resulted in a significant improvement of the extraction efficiencies for almost all FQs when compared to conventional SPE. Intra- and inter-day assays showed coefficients of variation below 10%. The better cleanup also resulted in the appearance of less precipitated matter in the final eluate, as well as reduced matrix effects. The results showed that the electrophoretic forces derived from electric fields are a promising way of significantly increasing the extraction efficiency of ionic analytes, while minimizing matrix effects associated with complex samples.

1. Introduction

An important analytical challenge that frequently needs to be resolved concerns interferences due to the complexity of many sample matrices, especially in the presence of low concentrations of the analytes of interest [1,2]. The rapid development and enhancement of modern analytical instruments has made it possible to improve the sensitivity and detection ability of many techniques. However, sample cleanup and preconcentration can still be problematic in solid phase extraction (SPE) procedures. In its first decades of use, SPE was extensively employed as a powerful sample preparation method that could be used for samples with complex matrices, such as biological fluids, tissues, foods, soils, and others [1–3]. Although SPE has many demonstrable advantages over conventional liquid–liquid extraction procedures, difficulties related to unsatisfactory cleanup and/or extraction efficiencies have persisted. These problems can be further aggravated in situations where analytes of interest that possess different physicochemical properties are present in the same sample, when losses of analytes and/or the co-extraction of interferents commonly occur.

Sample preparation techniques that employ electric fields as additional driving forces constitute one of the most recent approaches used to improve selectivity and extraction efficiency [4–7]. The use of electric fields is compatible with several sample preparation techniques, especially the two most widely used: solid phase extraction (SPE) and liquid–liquid extraction (LLE) [6]. Although electric field-assisted solid phase extraction has been widely adopted in on-line and in-line capillary electrophoresis, the approach using commercial syringe cartridges, called E-SPE, was only recently described [8,9]. Different to in-line and on-line solid phase extraction capillary electrophoresis, in E-SPE the chromatographic mechanisms (convection flow by pressure difference and sorption) form the basis of the technique, with the electrophoresis components (electroosmotic flow and electrophoretic velocities) providing auxiliary co- or contra-flow forces that can significantly influence the overall flow [8,9].

Another two phenomena that have already been described in electrophoresis (including capillary electrochromatography and capillary electrophoresis) are concentration polarization and Joule heating [10,11], both of which can significantly change the distribution constant of the analyte between the eluent and the sorbent. The use of such E-SPE electrophoretic phenomena during sample cleanup, in association with conventional chromatography sorption mechanisms, can lead to substantial improvements in selectivity and extraction efficiency, compared to conventional SPE.

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Eggs are complex matrices and residues of quinolones (in the ng g^{-1} concentration range) can be present, especially when good management and nutritional practices are not implemented in poultry production [12]. Conventional liquid–liquid extraction, solid phase extraction, and matrix solid phase dispersion are normally used as sample preparation techniques for the determination of quinolones in eggs [13].

In this work, electric fields were used for the first time during the solid phase extraction of four fluoroquinolones (FQs) from egg samples, with application of the fields during the washing and final elution steps. This strategy enabled the use of strong eluents during the washing step and weaker eluents during the final elution, improving the cleanup without compromising the extraction efficiency, prior to analysis using UHPLC–MS/MS.

2. Materials and methods

2.1. Chemicals and reagents

Ciprofloxacin (CIP), danofloxacin (DAN), and enrofloxacin (ENR) were all purchased from Fluka (Steinheim, Switzerland). Sarafloxacin (SAR) was purchased from Riedel-de Haën (Seelze, Germany). All the FQ standards showed purity higher than 95%. The chemical structures and p*K*a values of the antimicrobials used as model compounds are provided in the Supplementary Data (S1).

The analytical grade reagents disodium hydrogen phosphate dodecahydrate, citric acid monohydrate, sodium chloride, and sodium dodecyl sulfate were purchased from Merck (Darmstadt, Germany). Methanol, acetonitrile, and formic acid (liquid chromatography grade) were also supplied by Merck, and acetic acid was from Synth (Diadema, SP, Brazil). Throughout the studies, high purity water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Buffer solutions

Citric acid–phosphate buffers (CPBs) were used in this study, as described by McIlvaine [14]. The CPBs were prepared by mixing appropriate quantities of citric acid and disodium hydrogen phosphate solutions to obtain solutions with a final pH of 8.0. Two different CPBs were prepared. For the first (denoted CPB 10/20), 10 mmol L^{-1} citric acid and 20 mmol L^{-1} disodium hydrogen phosphate were mixed until pH 8.0 was reached. For the second buffer solution (denoted CPB 100/200), 100 mmol L^{-1} citric acid and 200 mmol L^{-1} disodium hydrogen phosphate were mixed until pH 8.0 was reached.

2.3. Standard solutions and calibration curves

A stock solution of each FQ (CIP, ENR, SAR, or DAN) was prepared separately in methanol:acetic acid (98:2, v/v) at a concentration of 40 μg mL^{-1}. The four stock solutions were diluted and mixed together to prepare a working standard solution at a concentration of 1000 ng mL^{-1} in methanol:acetonitrile:water: formic acid (11:4:84.15:0.85, v/v/v/v). The stock and working solutions were kept at −18 and 4 °C, respectively. The working standard solution was used to construct the matrix matched calibration curves for all analytes by spiking blank egg samples at five concentration levels (25, 50, 100, 150, and 200 ng g^{-1}).

2.4. Egg sample preparation

Eggs were obtained in a local market in Campinas (São Paulo State, Brazil) and were certificated as being from an organic farming system. The whole eggs (yolk and albumen) were homogenized using an Ultra Turrax® (Staufen, Germany), and 2 g portions were weighed out into 50 mL polypropylene tubes.

2.5. Extraction procedure

The egg sample preparation consisted of two stages: protein precipitation, and solid phase extraction with or without electric fields (E-SPE or SPE). Portions (2 g) of the homogenized whole eggs were fortified with the working FQ solution at the desired concentration. The proteins were then precipitated by adding 4 mL of acetonitrile containing 1% of acetic acid (v/v), followed by centrifuging at 4186 *g* for 5 min, as described previously [15]. After precipitation of the protein, the supernatant was evaporated to dryness under a flow of nitrogen at 40 °C, and the residue was resuspended in 2 mL of CPB 100/200 buffer at pH 8.0.

Cleanup of the sample extract was performed by E-SPE or SPE. The flow rates used during the introduction of the sample into the cartridge, the washing step, and the elution step were 3.0, 1.0, and 0.6 mL min^{-1}, respectively.

The solid phase extraction consisted of: (1) conditioning the cartridge with 3 mL of methanol, 3 mL of water, and 3 mL of CPB 100/200 pH 8.0 buffer; (2) application of the egg sample extract obtained after protein precipitation (residue resuspended in 2 mL CPB 100/200 pH 8.0 buffer); (3) addition of 700 μL of CPB 10/20 pH 8.0 buffer; (4) cleanup with 2.5 mL of CPB 10/20 pH 8.0 buffer containing 2% of acetonitrile (v/v); and (5) eluting with 3 mL of CPB 10/20 pH 8.0 buffer containing 15% of tetrahydrofuran (v/v). The final eluate was evaporated to dryness under a nitrogen flow at 40 °C, and the residue was dissolved in 0.5 mL of methanol: acetonitrile:water:formic acid (11:4:84.15:0.85, v/v/v/v) before analysis by UHPLC–MS/MS.

2.6. E-SPE cartridges and flow extraction system

Conventional empty polypropylene SPE cartridges (6 mL capacity) and polytetrafluoroethylene (PTFE) frits, both obtained from Varian (Palo Alto, CA, USA) were used to assemble the E-SPE cartridges. The sorbent consisted of 500 mg of Strata X® (33 μm average particle diameter, 90 Å pore size), purchased from Phenomenex (Torrance, CA, USA). The two electrodes, composed of stainless steel grids (120 mesh) and spiral-shaped wires, were arranged as described previously [9].

A semi-automated extraction system [16] was employed to control the electric potential and the flow rate, as well as to monitor the electric current during the extraction procedure. The original semi-automated system was modified with a solid-state relay that automatically turned the electric potential on and off during the E-SPE procedures (Fig. 1).

2.7. Comparison between E-SPE and SPE

The two different extraction methods (E-SPE and SPE) were compared in terms of the extraction efficiency and cleanup results. In the first method (E-SPE), electric fields were applied during the washing step (using a potential of 30 V, with the top electrode as the anode) and during the elution step (using a potential of 48 V, with the top electrode as the cathode). The second method (SPE) was a conventional solid phase extraction, without an electric field in any step. The extraction efficiencies were calculated using blank egg samples spiked with the FQs at concentrations of 100 ng g^{-1}. The results were presented as the averages of analyses performed in triplicate ($n=3$).

The cleanups achieved with the E-SPE and SPE procedures were compared in terms of the magnitude of the matrix effect (ME) observed in quantification of the FQs by UHPLC-MS/MS according to Eq. (1) [17]: