Analytica Chimica Acta 935 (2016) 269–281

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Innovative combination of QuEChERS extraction with on-line solid-phase extract purification and pre-concentration, followed by liquid chromatography-tandem mass spectrometry for the determination of non-steroidal anti-inflammatory drugs and their metabolites in sewage sludge

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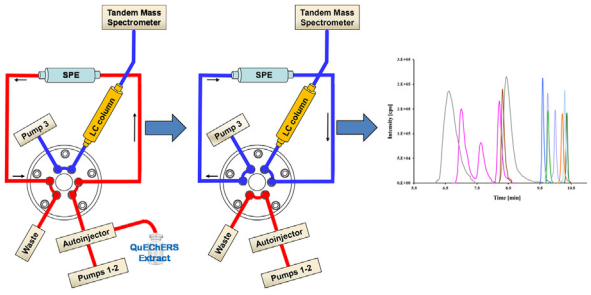
HIGHLIGHTS

- Non-steroidal anti-inflammatory drugs and their metabolites are analysed in sludge.
- QuEChERS extract is automatically preconcentrated, purificated and analysed by LC-MS.
- In most cases matrix effect was ≤20% and recovery ≥50%.
- The determination of target analytes in sludge is achieved in 30 min.
- The method sensitivity is high, being it from tens of pg g^{-1} to ng g^{-1} of dry sludge.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 March 2016
Received in revised form
10 June 2016
Accepted 19 June 2016
Available online 21 June 2016

Keywords:
QuEChERS
Solid-phase pre-concentration and purification
Liquid chromatography-tandem mass spectrometry
Sewage sludge
Non-steroidal anti-inflammatory drugs
Drug metabolites

ABSTRACT

For the first time QuEChERS extraction of sewage sludge was combined with the automatic solid-phase pre-concentration and purification of the extract (following indicated as SPE) and LC-MS/MS analysis, for the determination of the non-steroidal anti-inflammatory drugs acetylsalicylic acid (ASA), diclofenac (DIC), fenbufen (FEN), flurbiprofen (FLU), ketoprofen (KET), ibuprofen (IBU) and naproxen (NAP), and their metabolites salicylic acid (SAL), 4′-hydroxydiclofenac (4′-HYDIC), 1-hydroxyibuprofen (1-HYIBU), 2-hydroxyibuprofen (2-HYIBU), 3-hydroxyibuprofen (3-HYIBU) and *o*-desmethylnaproxen (O-DMNAP). Various commercial pellicular stationary phases (i.e. silica gel functionalized with octadecyl, biphenyl, phenylhexyl and pentafluorophenyl groups) were preliminarily investigated for the resolution of target analytes and different sorbent phases (i.e. octyl or octadecyl functionalized silica gel and a polymeric phase functionalized with *N*-benzylpyrrolidone groups) were tested for the SPE phase. The optimized method involves the QuEChERS extraction of 1 g of freeze-dried sludge with 15 mL of water/acetonitrile 1/2 (*v*/*v*), the SPE of the extract with the *N*-benzylpyrrolidone polymeric phase and the water/acetonitrile gradient elution on the pentafluorophenyl stationary phase at room temperature. Matrix effect was always suppressive and in most cases low, being it ≤20% for ASA, DIC, FLU, KET, IBU, 1-HYIBU, 2-HYIBU, 3-HYIBU, NAP and O-DMNAP, and included in the range of 35–47% for the other analytes. Recoveries were

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http://dx.doi.org/10.1016/j.aca.2016.06.023

evaluated at three spiking levels, evidencing almost quantitative values for HYIBUs and O-DMNAP; for ASA, SAL and KET the recoveries were included in between 50 and 76%, whereas for the other compounds they ranged from 36% to 55%. The proposed method showed better analytical performances than those so far published, being suitable for target compound determination in real samples from tens of pg g^{-1} to ng g^{-1} of freeze-dried sludge, with a total analysis time of 30 min per sample.

1. Introduction

Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method is an extraction and clean-up technique originally developed for recovering pesticide residues from fruits and vegetables [1–3] and thereafter applied to the analysis of various organic micropollutants in different environmental matrices, mainly of solid nature, such as sediments and soil [4].

The recovery from soil of selected drugs and herbicides, characterized by low values of the octanol-water partition coefficient (i.e. log K_{OW} = 0.8–2.8), has been demonstrated by the QuEChERS method [5], thus suggesting its suitability also for a wide range of polar compounds, including pharmaceuticals and their metabolites.

The determination of organic micropollutants in sewage sludge is surely a topic of great interest from an environmental point of view. In fact, biological sludge may represent the final sink of organic micropollutants in wastewater treatment plants (WWTPs), the determination of which can give useful information concerning the overall efficiency of the wastewater treatment process, as well as the potential soil contamination, when these bio solids are used for land applications [6,7].

Organic micropollutants in sewage sludge have been analysed using different recovery techniques such as pressurized liquid extraction and microwave assisted extraction, usually followed by SPE clean-up procedures [8]. Instrumental determination of organic analytes in sludge has been performed by using both gas chromatography (GC) and liquid chromatography (LC), depending on the physicochemical characteristics of investigated compounds, mainly coupled with mass spectrometry (MS) [8]. However, in this regard, it should be emphasized that, when thermally labile and/or polar analytes like pharmaceuticals have to be analysed, a proper derivatization step is necessary for GC analysis [9,10], whereas their direct determination can be performed in LC.

Among the various extraction techniques applied to the recovery of organic micropollutants from biological sludge, the QuEChERS approach was seldom adopted. To date, these studies focus on the determination of selected benzotriazole, benzothiazole and benzenesulfonamide derivatives [11], and a number of hormones, pharmaceuticals and personal care products [12–14]. In these works the traditional QuEChERS extraction and dispersive solid-phase (d-SPE) purification of the extract, based on the use of "primary secondary amine" (PSA) as sorbent, was coupled with liquid chromatographic (LC) analysis and tandem mass spectrometric (MS/MS) [11,12,14] or single time of flight mass detection [13].

Even though the QuEChERS technique can be considered as a high-throughput analytical approach, the d-SPE step significantly increases the analysis time and involves an extra sample manipulation, compared to the extraction alone. Moreover, large matrix effects (ME) have been often observed, especially when ESI-MS detection is employed, notwithstanding various d-SPE sorbents, besides PSA, were investigated to lower the matrix influence [13]. A remarkable decrease in total analysis time, together with a significant increase of the overall pre-concentration factor, would be achieved by treating the QuEChERS extract like a water sample, by the on-line solid-phase pre-concentration and purification technique, automatically coupled with LC-MS/MS (on-line SPE-LC-MS/MS), which has been extensively applied to the determination of various classes of organic micropollutants in environmental waters [15–17].

Accordingly, the aim of this research was to investigate the combination of QuEChERS extraction with on-line SPE-LC-MS/MS for the determination of selected pharmaceutical compounds in sewage sludge. More in detail, various commercially available sorbent phases (i.e. silica gel functionalized with octyl or octadecyl groups and a polymeric phase functionalized with *N*-benzylpyrrolidone groups) were evaluated for replacing the d-SPE step of the QuEChERS method. Furthermore, some analytical stationary phases (i.e. silica gel functionalized with octadecyl, biphenyl, phenylhexyl and pentafluorophenyl groups), characterized by different physicochemical properties, were tested.

Target compounds of this study (i.e. acetylsalicylic acid, diclofenac, fenbufen, flurbiprofen, ibuprofen, ketoprofen and naproxen) were chosen within the group of non-steroidal anti-inflammatory drugs (NSAIDs), which represent one of the most worldwide consumed class of pharmaceutical compounds [18–20]. Furthermore, some NSAIDs (e.g. diclofenac and ibuprofen) are characterized by endocrine disruption properties [21,22] and have been previously found in biological sludge [10,13,23]. It should also be noted that some NSAID metabolites (i.e. salicylic acid, 4′-hydroxydiclofenac, 1-hydroxyibuprofen, 2-hydroxyibuprofen, 3-hydroxyibuprofen and O-desmethylnaproxen), never investigated before in sewage sludge, were included in this study. Target analytes were characterized by a very wide range of polarity (log K_{OW} included in the range 1.4–4.5), thus representing a group of chemicals very interesting to be studied from an analytical viewpoint during the various partition steps involved in both the QuEChERS and the SPE phases.

2. Experimental

2.1. Chemicals and materials

LC–MS grade methanol, acetonitrile, water, formic acid, HPLC grade methanol and acetonitrile were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ultrapure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA). Sodium chloride and magnesium sulphate heptahydrate used for QuEChERS extraction were obtained from Sigma–Aldrich.

Acetylsalicylic acid (ASA, CAS: 50-78-2), acetylsalicylic acid D3 (ASA D3, CAS: 921943-73-9), salicylic acid (SAL, CAS: 69-72-7), diclofenac (DIC, CAS: 15307-79-6), diclofenac D4 (DIC D4, CAS: 153466-65-0), 4′-hydroxydiclofenac (4′-HYDIC, CAS: 64118-84-9), fenbufen (FEN, CAS: 36330-85-5), flurbiprofen (FLU, CAS: 5104-49-4), ketoprofen (KET, CAS: 22071-15-4), ketoprofen D3 (KET D3, CAS: 159490-55-8), ibuprofen (IBU, CAS: 15687-27-1), ibuprofen D3 (IBU D3, CAS: 121662-14-4), 1-hydroxyibuprofen (1-HYIBU, CAS: 53949-53-4), 2-hydroxyibuprofen (2-HYIBU, CAS: 51146-55-5), 3-