Analytica Chimica Acta xxx (2018) 1–10

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Automation of ionic liquid enhanced membrane bag-assisted-liquid-phase microextraction with liquid chromatography-tandem mass spectrometry for determination of glucocorticoids in water

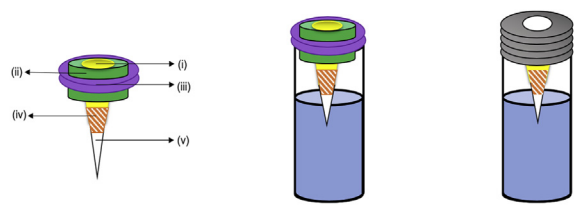
Shalene Xue Lin Goh [a, b], Hong Aik Goh [a], Hian Kee Lee [a, b, c, *]

[a] Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore
[b] National University of Singapore Environmental Research Institute, T-Lab Building #02-01, 5A Engineering Drive 1, Singapore 117411, Singapore
[c] Tropical Marine Science Institute, National University of Singapore, S2S Building, 18 Kent Ridge Road, Singapore 119227, Singapore

HIGHLIGHTS

- Fully automated membrane bag-liquid-phase microextraction was successfully enabled.
- A membrane bag was used as a novel extraction device.
- *n*-Octanol/ionic liquid extractant phase demonstrated good extraction performance.
- Good analytical performance was achieved for glucocorticoids.
- Only low sample volumes were required with minimal sample clean-up.

GRAPHICAL ABSTRACT



(a) Final extraction device: (i) supporting device 1 (modified 200 μL pipette tip); (ii) supporting device 2 (modified polypropylene insert and 2 mL Eppendorf tube); (iii) supporting device 3 (rubber septum from the cap of 10 mL vial); (iv) Parafilm; (v) membrane bag.

(b) Cross-sectional diagram of the extraction device resting on the neck of the vial containing sample solution.

(c) Extraction device in the vial containing sample solution (with the metal cap).

ARTICLE INFO

Article history:
Received 12 May 2018
Received in revised form
13 July 2018
Accepted 16 July 2018
Available online xxx

Keywords:
Membrane bag-assisted liquid-phase microextraction
Ionic liquid
Automation
Glucocorticoids
Ultra-high performance liquid chromatography-mass spectrometry

ABSTRACT

A novel fully automated liquid-phase microextraction (LPME) procedure making use of a conical polypropylene membrane bag to hold the solvent, coupled with ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) with positive electrospray ionisation was developed to determine glucocorticoids (including cortisol, cortisone, dexamethasone, prednisone and prednisolone) in water. The solvent was a synergistic mixture of 10% v/v of ionic liquid, 1-butyl-3-methylimidazolium methylsulfate in *n*-octanol. The use of ionic liquid as an additive enhanced the extraction performance due to the favourable ionic and hydrogen bonding interactions with the analytes. Different experimental parameters such as the types of organic solvent as supported liquid membrane and ionic liquid, various composition of ionic liquid, volume of extractant phase, agitation time and speed, temperature of extraction were investigated. Under the most favourable extraction conditions, enrichment factors of 49.4–83.1 were obtained for the target compounds with relative standard deviations of <10%. The intra-day repeatability of the method ranged from 4.23 to 6.42% and the inter-day reproducibility ranged from 6.87 to 9.20%. Good linearity 0.05–50 ng mL^{-1} (prednisolone) and 0.1–50 ng mL^{-1} (all other analytes) with coefficients of determination of 0.991 or better, was obtained. The membrane bag-assisted-LPME UHPLC-MS/MS approach exhibited high sensitivity, linearity and repeatability for the extraction of the glucocorticoids and also offered an automated streamlined process, from the point where analytes were extracted, to the final analysis of the water samples. The method was

* Corresponding author. Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore.
E-mail address: chmleehk@nus.edu.sg (H.K. Lee).

https://doi.org/10.1016/j.aca.2018.07.031

employed to determine the concentration of these contaminants in the influent and effluent of a wastewater treatment plant.

1. Introduction

Increasing recognition of the potential harmful effects posed by glucocorticoids has provided the impetus for research into the presence of these compounds in the environment, in recent years [1–4]. Glucocorticoids are produced by the adrenal cortex, and are involved in regulating the biosynthesis and metabolism of carbohydrates, fats and proteins. Natural and synthetic glucocorticoids are often prescribed as medication for immune-mediated conditions or as anti-inflammatory agents [5,6], and are administered through oral, or intraveneous means, or external application.

The excretion of pharmaceutical residues by humans and animals in their free forms or derivatives [7] represents the major source of these chemicals as contaminants entering the aquatic environment. Glucocorticoids, that are synthesised for external application, are usually of particularly higher stability and have higher residual rates in the environment (compared to those administered through oral and intraveneous means) as the residues found on the skin and clothing are eventually washed into the ecosystem without being metabolised [7]. Schriks et al. reported considerably high concentration of glucocorticoid residues in different types of wastewater, especially in hospital discharges [2]. Nevertheless, current treatment processes in many wastewater treatment plants (WWTPs) are ineffective in the thorough removal of these contaminants [8]. This can lead to their discharge into the marine ecosystem via the effluent of WWTPs [3] and bring about potentially adverse effects on organisms. For instance, studies have demonstrated that chronic exposure to cortisol (CORS) can cause fishes to experience adverse effects in their movements, inhibiting antagonistic behavior and changes in their sexual behavior and immune responses [9,10]. It is therefore essential to develop sensitive analytical methods to detect and monitor glucocorticoids in aqueous matrices to allow preventive measures to be implemented in time.

There are limited sample preparation studies on glucocorticoids in environmental water [2–4,7,8,11–16], especially of microextraction techniques [4,17]. The latter offer added advantages over the traditional methods of solid-phase extraction (SPE) and liquid-liquid extraction (LLE) which have generally been used to preconcentrate glucocorticoids in the past decade. Microextraction procedures, of which liquid-phase microextraction (LPME) is an example, have advantages over LLE, such as lower solvent consumption, and high preconcentration factors and extraction efficiencies.

There are various LPME modes, including one in which hollow-fiber membrane (HF) [18–20] is used as a protective sheath for the solvent, against potential interfering substances in the sample matrix. The extraction device is also disposed of after each extraction, thereby eliminating cross contamination between samples. Although automated tubular HF-LPME [21–24] and automated membrane bag-LPME [25] (as membrane-assisted solvent extraction) have been introduced, neither approach has been widely practiced subsequently, probably because of the difficulties in the automated microsyringe needle operations involving narrow-bore (usually <1 mm) HFs, or the unsatisfactory enrichment limited by the large (800 μL) commercially available membrane bags. Notwithstanding the latter, the use of a membrane bag is still preferable, given its greater flexibility since it can hold a larger volume of solvent for greater extraction capacity (subject to the dilution effect coming into effect), and it also allows a greater margin of error in managing microsyringe needle movements; this is the approach adopted in the present work.

Ionic liquids at room temperature are salts that demonstrate good stability in air and water, and are composed of organic cations and various anions. They possess low volatility, high thermal stability, and are also considered as environmentally friendly solvents. In addition, they can enhance selectivity and improve the kinetic constant of reaction as they are able to stabilise any charged intermediates formed in the process [18,26]. Ionic liquids have found numerous uses in the extraction of steroidal compounds [4,27,28]. However, they are generally expensive and highly viscous. For example, 1-butyl-3-methylimidazolium methylsulfate has a viscosity of 173.5 cP at 303 K while that of an organic solvent, *n*-octanol, is only 6.247 cP at the same temperature. Pure ionic liquids are generally too viscous to homogenously penetrate and saturate the surface of the polypropylene membrane sheet. According to Fick's first law, the rate of diffusion of the analytes into the extractant phase is directly proportional to the concentration gradient, and has an inverse relation to the diffusion coefficients of the analytes in the particular medium (which are in turn dependent on the temperature, the viscosity of the medium, and the size of diffusing particles). A viscous extractant solvent is associated with a poorer diffusion rate of the analytes into it, leading to lower enrichment. To overcome the viscosity issue, ionic liquid may be mixed with organic solvents.

In this study, the possibility of employing a synergistic mixture of *n*-octanol and ionic liquid was explored as an extractant solvent for glucocorticoids. An additional objective of this study, as mentioned above, was also to develop an automated extraction method with the use of a polypropylene membrane bag filled with an organic solvent-ionic liquid mixture for the LPME of the glucocorticoids, followed by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) under positive electrospray ionisation mode (ESI). The method was applied to determine glucocorticoids in water samples from a WWTP in Singapore.

2. Experimental

2.1. Reagents and materials

High purity standards for cortisone (CORT), dexamethasone (DEXA), prednisone (PRD), prednisolone (PRNL) and CORS were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Their chemical structures are presented in Table 1. 1-Butyl-3-methylimidazolium methylsulfate ([BMIM]$MeSO_4$; 98%), 1-butyl-3-methylimidazolium tetraborofluorate ([BMIM]BF_4; 98%) and 1-butyl-3-methylimidazolium chloride ([BMIM]Cl; 98%) were purchased from Strem Chemicals (Newburyport, MA, USA). 1-Butyl-3-methylimidazolium hexafluorophosphate ([BMIM]PF_6; 98%) was obtained from Apollo Scientific (Stockport, Cheshire, United Kingdom). 1-Butyl-1-methylpyrrolidinium tris(pentafluoroethyl) trifluorophosphate ([BMPL]TFP; 98%) and 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate ([HMIM]TFP; 98%) were procured from Merck Chemicals (Darmstadt, Germany). 1-Octylamine and 1-octanoic acid were obtained

Please cite this article in press as: S.X.L. Goh, et al., Automation of ionic liquid enhanced membrane bag-assisted-liquid-phase microextraction with liquid chromatography-tandem mass spectrometry for determination of glucocorticoids in water, Analytica Chimica Acta (2018), https://doi.org/10.1016/j.aca.2018.07.031