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Sample-preparation methods for direct and indirect analysis of natural estrogens

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ABSTRACT

Estrogen conjugates can be analyzed directly or indirectly using different sample-preparation methods. This review first summarizes the sample-preparation method for direct analysis; then indirect analysis by enzymatic-hydrolysis and chemical-deconjugation methods. We discuss progress and challenges for sample-preparation methods. Overall, sample preparation for direct analysis represents the latest development, while we describe the most widely employed method, enzymatic hydrolysis, as the limiting step in sample analysis due to long incubation times. Although the chemical deconjugation is currently the least used method, its potential is probably underestimated. A recently developed acid-solvolysis method may be widely applied to environmental samples for its high deconjugation efficiency and because it is more cost effective. This method can possibly extend to biological samples, although great efforts must be made to validate its feasibility.

Contents

1. Introduction

In the past few decades, endocrine-disrupting compounds (EDCs) have been extensively studied for their possible adverse effects on the environment [1]. EDCs have the capacity to elicit negative effects on the endocrine systems of both humans and wildlife [1]. With increasing understanding of EDCs, the term now covers a broad class of chemicals, including natural estrogens, androgens, phytoestrogens, synthetic estrogens and androgens, mycoestrogens and industrial chemicals [1,2]. Among these, natural estrogens E_1, E_2, and E_3 are viewed as core EDCs, and are the most commonly studied and monitored. Based on a summary of data on human urinary excretion, Liu et al. [3] concluded that natural estrogens other than E_1, E_2, and E_3 should also be considered important EDCs.

The main routes of excretion are via urine and feces, which are the main sources of these compounds in wastewaters. While most natural estrogens excreted in urine are estrogen conjugates, the majority of these conjugates were thought to be transformed into their corresponding free estrogens before their entry into sewage-treatment plants [4]. However, a simulation batch study showed that

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complete cleavage of estrogen glucuronides in wastewater took more than 20 h, while cleavage of estrogen sulfates was even slower, taking more than 140 h [5]. These experimental results suggest that a portion of estrogen conjugates exist in the sewage of wastewater-treatment plants. This has been proved in a few on-site investigations [5–7]. Estrogen conjugates are very weakly estrogenic, but, as they can be cleaved into strongly estrogenic free estrogens, their adverse effects are similar to those of their free estrogens. It is therefore necessary to monitor both free and conjugated estrogens in wastewater. While routine analysis of these compounds in wastewater is time consuming and very expensive, estimation of possible concentrations based on data about excretion of urine and feces is necessary and cost effective. For effective estimation, accurate measurement of natural estrogen conjugates in samples of urine and feces is crucial. In addition, routine analyses of natural estrogen conjugates in biological samples are common, due to the link between estrogen-excretion rates and human disease.

There are two routinely used classes of analytical methods for estrogen conjugates, direct and indirect, as shown in Fig. 1. Due to the hydrophilic properties of estrogen conjugates, preparation of environmental or biological samples is very complex. The objectives of this review are to summarize:

(1) sample-preparation methods of estrogen conjugates for direct analysis;
(2) sample-preparation methods of estrogen conjugates for indirect analysis, including enzymatic hydrolysis and chemical deconjugation; and,
(3) the progress and the challenges associated with these sample-preparation methods.

2. Basic properties of estrogen conjugates

Currently, 16 natural estrogens have been detected in human urine [3,8]. Another natural estrogen, estetrol, is detectable in pregnant women only [9]. As the functional groups of sulfate and glucuronide can bind to estrogens in several different places, several times more natural conjugates exist than free estrogens [3]. However, estrogen conjugates are far less studied than free estrogens, and only a small amount have been directly quantified without deconjugation, and they are limited to the family of E_1, E_2, and E_3. The basic properties of these estrogen conjugates are summarized in Table 1, in comparison with their corresponding free estrogens. Most estrogen conjugates in biological samples are first deconjugated by enzymatic hydrolysis or chemical-deconjugation methods, and quantified as the total corresponding free estrogens [3,8].

3. Sample preparation for direct and indirect analyses

Analytical methods of estrogen conjugates are actively being developed and can be classed as direct and indirect determinations. Historically, the predominant method was indirect, in which conjugates were first cleaved to their corresponding free estrogens before determination. However, with the great progress of chemical analysis, direct analysis of estrogen conjugates with liquid chromatography tandem mass spectrometry (LC-MS/MS) has become possible for both environmental and urine samples [15,16]. Direct analysis of these conjugates is more desirable, as we can know exactly which conjugates exist in monitored samples and their exact concentrations, while the indirect methods can only roughly differentiate between sulfates or glucuronide conjugates. Although there are disadvantages to indirect analytical methods, it seems impractical to cover all possible estrogen conjugates for direct analysis with LC-MS/MS, especially on the massive scale necessary, so the indirect analytical methods are still indispensable.

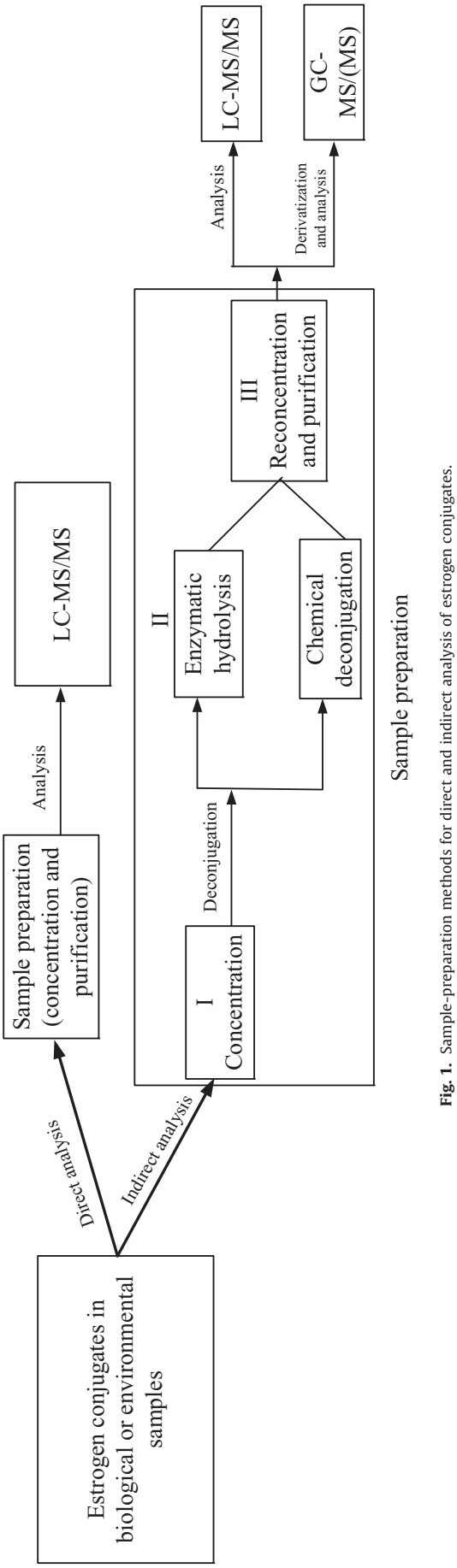

Fig. 1. Sample-preparation methods for direct and indirect analysis of estrogen conjugates.