Journal of Chromatography A, 1280 (2013) 84–91

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Analysis of estrogenic compounds in environmental and biological samples by liquid chromatography–tandem mass spectrometry with stable isotope-coded ionization-enhancing reagent

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ARTICLE INFO

Article history:
Received 29 July 2012
Received in revised form 9 January 2013
Accepted 10 January 2013
Available online 16 January 2013

Keywords:
Estrogenic compounds
Stable isotope labeling
LC–MS/MS
Matrix effects

ABSTRACT

A sensitive and reliable stable isotope labeling technology was developed for the determination of estrogenic compounds in environmental and biological samples based on the derivatization of estrogenic compounds with 10-methyl-acridone-2-sulfonyl chloride (d_0-MASC) and its deuterated counterpart d_3-MASC. The labeling reaction of MASC with estrogenic compounds is simple and robust and can be carried out under mild conditions within 5 min. Internal standard-based quantification was achieved by this labeling strategy without the need of using expensive internal standard analogy to every analyte of interest. Meanwhile, the sensitivity obtained by liquid chromatography–tandem mass spectrometry (LC–MS/MS) was enhanced by 2–3 orders of magnitude compared to the underivatized counterparts. Application of the stable isotope labeling technology in relative and absolute quantification of estrogenic compounds in complicated samples indicated that the labeling strategy was effective in overcoming matrix effects. The proposed method was successfully applied to the analysis estrogenic compounds in different environmental and biological samples with high sensitivity and accuracy.

1. Introduction

Mass spectrometry (MS) is highly popular because of its high sensitivity and specificity compared to other analytical techniques. The hyphenation of MS to liquid chromatography (LC–MS) is especially popular due to its fast and sensitive characterization and quantification. Nowadays, LC–MS plays a growing important role in many fields [1–9]. However, endogenous matrix components may coelute with the analytes of interest and thus affect the reproducibility and accuracy of the method. In extreme cases, matrix components can cause ionization suppression to such an extent that the analytes are rendered undetectable by MS [10].

Great efforts have been made to reduce matrix effects [11,12]. Solid phase extraction (SPE) is one of the often used methods to minimize matrix effects. However, SPE methods are only moderately successful in overcoming matrix effect because it is difficult to remove matrix components possessing similar property to the analytes. These components are likely to coelute with the analytes in LC–MS and continue to cause ionization suppression [10]. Another often used strategy is to use stable isotope labeled (SIL) internal standard [13]. Matrix effects can be reduced to a minimal level since matrix effects observed for the SIL internal standard are generally similar to those observed for the analytes. However, only a limited number of SIL internal standards are commercially available, and it is expensive and not practical to synthesize SIL internal standard to every analyte of interest, especially when there are a variety of target compounds. Recently, a new strategy employing stable isotope labeling overcomes some of the drawbacks mentioned above and becomes popular in many fields [13–18]. Instead of synthesizing an isotope analogy of the analyte of interest, stable isotope labeling method uses a chemical reaction to introduce an isotope tag to the analyte in one sample and another mass-difference isotope tag to the same analyte in another comparative sample (or standard), followed by mixing the two labeled samples for mass spectrometric analysis. The isotopic pairs of the labeled analytes coelute within a single run and have identical retention times. Since they are electrosprayed from identical solution conditions, the matrix effects and ionization efficiencies are expected to be the same.

Estrogenic compounds have gained increasing environmental and social concerns in recent years because of their endocrine-disrupting property and other serious side effects on human health [19–23]. Various approaches have been developed for the

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http://dx.doi.org/10.1016/j.chroma.2013.01.045

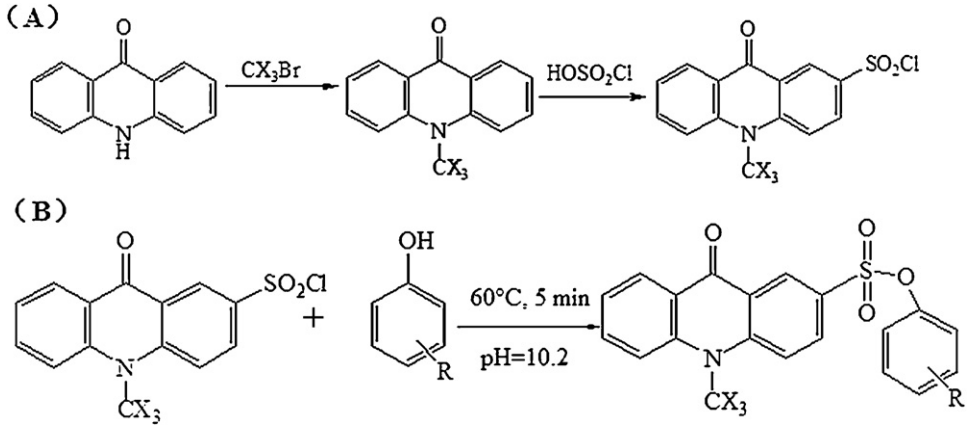

Fig. 1. Schemes of synthesis and derivatization. (A) Synthesis routes of d_0- and d_3-MASC. (B) Reaction scheme of MASC with estrogenic compounds. X = H or D.

determination of estrogenic compounds in these samples, such as liquid chromatography (LC) [24,25], LC–MS [26,27] and gas chromatography–mass spectrometry (GC–MS) [23,28]. However, many studies indicated that the application of these methods was often hampered by the low sensitivity or severe matrix effect [29–31].

In this work, we report a stable isotope labeling technology for improved quantification of estrogenic compounds in environmental and biological samples. Differential isotope labeling of estrogenic compounds with isotope-coded MASC provided isotopic variants which coeluted on a reversed-phase column. Matrix effects and run-to-run ionization differences which were often encountered in direct LC–MS analysis were therefore greatly reduced by the application of deuterated internal standard generated through derivatization. Meanwhile, the ionization efficiency of estrogenic compounds was greatly enhanced through the introduction of a readily ionizable MASC moiety into the analyte. The established method can be well applied to the relative and absolute quantification of unknown substances, such as phenolic- and amino-containing components, which can also react with MASC.

2. Experimental

2.1. Reagents and chemicals

Analytical standards of 4-octylphenol (OP), 4-nonylphenol (NP), bisphenol A (BPA), diethylstilbestrol (DES), estrone (E1), 17α-ethynylestradiol (EE2), 17β-estradiol (E2) and estriol (E3) were all obtained from Dr. Ehrenstorfer (Ausburg, Germany) with purity of higher than 99%. Methanol, dichloromethane, ethyl acetate, n-hexane and acetonitrile were of HPLC grade and purchased from Sigma–Aldrich (USA). Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents used were of HPLC grade or at least of analytical grade. ODS C18 cartridges (500 mg, 6 mL) were obtained from Chrome Expert (CA, USA).

Individual stock solutions of 100 mg/L for all compounds were prepared in HPLC-grade acetonitrile and stored at 4 °C in the dark. Standard solutions containing all compounds were mixed and diluted with acetonitrile, and working solutions of all compounds and calibration concentrations were prepared by appropriate dilution of the stock solutions on the day of analysis.

d_0-MASC or d_3-MASC were synthesized in authors' laboratory as described in the synthesis section and Fig. 1A. The derivatizing reagent solution (1.0×10^{-3} mol/L) was prepared by dissolving 3.1 mg MASC in 10 mL of anhydrous acetonitrile. When not in use, all reagent solutions were stored at 4 °C in a refrigerator.

2.2. Synthesis of d_0-MASC and d_3-MASC

The preparation of stable isotope labeling reagents was carried out by a two-step procedure similar to the previously described method in our laboratory [32]. In brief, to a 200-mL flask, potassium hydroxide (2.6 g) and DMSO (30 mL) were mixed at room temperature for 10 min. Then acridone (5.8 g) in 20 mL of DMSO solution was added and stirred at room temperature for 40 min. A solution of $^1H_3$-bromomethane or $^2H_3$-bromomethane (13 mL) in 5.5 mL of DMSO solution was then added dropwise within 10 min. The contents were kept at room temperature for 24 h with vigorous stirring. The reaction mixture was poured into 100 mL of water with vigorous stirring for 10 min. The precipitated solid was recovered by filtration, washed with water, and dried with P_2O_5 under vacuum for 24 h. The crude products were recrystallized three times from acetonitrile to afford a yellow crystal (10-methyl acridone), yield 5.4 g (86%). The final product of 10-methyl-acridone-2-sulfonyl chloride (MASC) was synthesized by the reaction of chlorosulfonic acid with 10-methyl acridone. The synthesis procedure was exactly the same as that described before [32]. The synthesis procedures of light d_0-MASC and heavy d_3-MASC are depicted in Fig. 1A. The previously reported 10-ethyl-acridone-2-sulfonyl chloride (EASC) was not applied in this method because the synthesis of its deuterated counterpart would be more expensive.

2.3. Sample extraction

To avoid the contaminations from sample analysis process, glass syringes and glass vessels were employed throughout the experiments to avoid the introduction of NP or BPA. Syringes and vessels were all rinsed sequentially with tap water, high-purity water and methanol prior to sample addition.

2.3.1. Wastewater samples

Wastewater samples were collected from three different sites of a small river to which domestic sewage was discharged. Samples were extracted according to the method described in our previous work [33]. Wastewater samples were filtered through 0.45 μm pore size cellulose filters to remove fine particles. They were adjusted to pH 3.0 with 6 M HCl solution. Then 200 mL of water samples were passed through the ODS C18 SPE cartridges previously conditioned with 5 mL of ethyl acetate, 5 mL of methanol, and 5 mL of water. After washing with 10 mL of methanol–water (1/9, v/v), the cartridges were dried under vacuum for 10 min, then the analytes were eluted with 5.0 mL of mixed solvent of n-hexane/dichloromethane (9/1, v/v) and 7.0 mL of ethyl acetate. The eluted solutions were