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Determination of parabens in domestic sewage by isotope-coded derivatization coupled with high performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

We have established an isotope labeling protocol for determining parabens in wastewater, based on a pair of isotope-coded derivatization reagents, by high performance liquid chromatography (LC) and electrospray mass spectrometry (ESI-MS). The pair of reagents used in our work were d_0-10-methylacridone-2-sulfonyl chloride (d_0-MASC, light form) and d_3-10-methylacridone-2-sulfonyl chloride (d_3-MASC, heavy form). d_0-MASC and d_3-MASC could easily derivatize parabens at 55 °C for 7.5 min in the mixture of bicarbonate and acetonitrile (1:1 *v*/*v*). It is shown that the derivatization offers 1–2 orders of magnitude signal enhancement over underivatized counterparts. The global isotope internal standard technology was employed for quantification analysis with d_3-MASC-paraben as internal standard for corresponding d_0-MASC-paraben. In addition, homologous internal standard was simultaneously used to correct derivatization processes. The obtained double internal standard calibration curves for parabens were linear over the range of 1–500 nmol L^{-1} ($r > 0.997$). The isotope-coded derivatization based quantification method was precise and practical with RSD ≤ 3.6% and accuracy ranged 96.5 to 104.7%. The proposed method has also been successfully applied to the quantification of parabens in domestic sewage samples with precisions ≤5.2% and recoveries ≥87.9%. Five kinds of paraben including methylparaben, ethylparaben, propylparaben, butylparaben, and benzylparaben were detected in the tested samples. The quantitative results indicated that parabens were at nmol L^{-1} level or lower, and ethylparaben were the most abundant, followed by methylparaben, propylparaben and butylparaben. It has been demonstrated that the isotope-coded derivatization reagents are useful for accurate quantification of analytes having target functional groups in real samples.

1. Introduction

Parabens are a group homologous *p*-hydroxybenzoates, normally including methylparaben, ethylparaben, propylparaben and butylparaben. They are the most common preservatives used in foods, cosmetics, beverages, and pharmaceuticals due to their relatively low toxicity and effective antimicrobial activity [1]. Although parabens are used widely, surprisingly little attention has been paid to their potential hazards. The Final Report on Safety Assessment of Parabens concludes that the concentrations of paraben preservatives used in cosmetic products are safe [2]. However, some papers have appeared recently which warn about the possibility of parabens acting as endocrine disruptors [3]. Prusakiewicz et al. doubted that parabens inhibit human skin estrogen sulfotransferase activity [4]. Oishialso reported that propylparaben adversely affects the hormonal secretion and the male reproduction [5]. Therefore, such compounds can be considered to be emerging pollutants and their determination is likely to become increasingly important. Therefore, it is imperative to establish a sensitive, simple and rapid method to monitor the preservatives [6–8]. High-performance gas chromatography (GC) [9–12], liquid chromatography (LC) [13–17] and capillary electrophoresis (CE) [18,19] are prime methods for determination of parabens. The approaches based on mass spectrometry (MS) to analyze parabens are accurate and sensitive [9–17]. However, there are also some problems in the MS analytical method. Matrix effect (i.e., ion suppression or enhancement) is a common problem in LC-MS analysis of organics, which may adversely influence the analytical sensitivity and reliability. Using appropriate internal standards is therefore needed to achieve good

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recoveries and accurate quantification in complex environmental matrices. Stable isotope-coded synthetic standards (isoforms of analytes) are frequently used in quantitative analysis. Those isotope-coded synthetic standards identical in structure to analytes of interest behave like the analytes themselves in analytical systems, except for the molecular mass. When added in known concentrations to a sample, they serve as internal standards that compensate for inter-analysis variations in sample manipulation and MS analysis. A limitation of this method is that isotope-coded synthetic standards are expensive and sometimes unavailable, especially in the analysis of large numbers of analytes.

It has been shown in proteomics that one way to deal with this problem is to label large number of peptides through derivatization of primary amines with stable isotope-coded derivatization reagents. Peptides in one sample are globally coded with a single isotope of the coding agent (light form) while those of a second sample are globally coded with a second isotope (heavy form). After the two samples are mixed, the relative amount of isotopically coded peptides can be determined in a single LC-MS analysis. In recent years, this technology has also been used to relative or absolute quantify carboxylic acids, amino compounds, phenol compounds and so on [20,21]. Li's group has developed a pair of $^{13}C/^{12}C$ isotope-labeled *p*-dimethylaminophenacyl bromide, which is used to profile carboxylic acids-containing metabolites by LC-MS [22]. Also they have reported a quantitative metabolome profiling technique based on differential $^{12}C/^{13}C$-isotope dansylation labeling and fast LC-MS [23]. L. M. Smith's group offers a powerful approach to achieve relative quantification of carboxylic acids metabolites by LC-MS using H/D isotopic variants of cholamine [24]; they also provide a relative quantification method of amine metabolites by LC-MS with $^{13}C/^{12}C$ isotopic labeled methyl acetimidate [25]. F. E. Regnier's group offers an enhanced LC-MS method for analysis of fatty acids in human serum samples by using 2-bromo-1-methylpyridininium iodide and H/D isotope-coded 3-carbinol-1-methylpyridininium iodide as derivatization reagents [26]; and they have also developed H/D isotopic labeled *N*-methyl-nicotinic acid *N*-hydroxysuccinimide to quantification estrogen metabolites in breast cancer serum samples [27]. Y.Q. Feng's group have developed H/D isotope-coded ω-broomoacetonylpyridinium beomide and ω-broomoacetonylquinolinium beomide to determine compounds containing carboxylic acids and thiols, respectively [28,29]. Y. L. Guo's group has reported a direct N-alkylpyridinium isotope-coded quaternization procedure for mild derivatization of cholesterol and fatty alcohols [30]. J. L. Zhang's group carried out a series of works on fatty acids submetabolome based on isotope-coded 2,4-bis(diethylamino)-6-hydrazino-1,3,5-triazine [31,32]. X.X. Wang et al. proposed H_3/D_3-isotope-coded 4-(1-methyl-1H-phenanthro[9,10-*d*]imidazol-2-yl)phenlamine to determine aldehydes in aquatic products by liquid chromatography–tandem mass spectrometry [33]. In these works, the matrix effect during ionization process can be well calibrated by using isotope-coded derivatives as internal standards. However, we noticed that only a few groups have investigated the difference of derivatization yields caused by sample matrix between light and heavy systems [34–37].

In our previous works, The H_3/D_3 isotope-coded 10-methylacridone-2-sulfonyl chloride (MASC) were reported, and it has been successfully applied for determination of endocrine disrupting chemicals, amino acids and biogenic amines [38–40]. In this work, its application for determining parabens in wastewater samples has also been carried out by LC-MS combined with isotope-coded derivatization technique. We employed a double internal standard calibration method to carry out absolute quantification of parabens, and both ionization and derivatization processes were effectively corrected by simultaneously using homologous internal standard and isotope labeled internal standard. It has been demonstrated that the proposed reagents are of great value for determining compounds containing target functional groups of interests.

2. Experimental

2.1. Chemicals

10-Methyl-acridone-2-sulfonyl chloride (d_0-MASC) and d_3-10-methyl-acridone-2-sulfonyl chloride (d_3-MASC) was synthesized in our laboratory. Parabens standards including methylparaben (MP), ethylparaben (EP), propylparaben (PP), butylparaben (BP), and benzylparaben (BzP) and pentylparaben (PtP) were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). HPLC grade acetonitrile (CH_3CN) was purchased from Yucheng Chemical Reagent Co. (Shandong Province, China). Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents were of analytical grade unless otherwise stated.

2.2. Preparation of solutions

Individual stock solutions (1.0×10^{-2} mol L^{-1}) of the parabens were prepared in acetonitrile. The standard parabens for HPLC analysis at individual concentrations of 5.0×10^{-5} mol L^{-1} were prepared by diluting the corresponding stock solutions (1.0×10^{-2} mol L^{-1}) with acetonitrile. The d_0-MASC and d_3-MASC solution (1.0×10^{-3} mol L^{-1}) was prepared by dissolving 19.2 mg solides in 10 mL of acetonitrile, respectively. Sodium bicarbonate buffers (0.1 mol L^{-1}) were adjusted to pH 9.58 with sodium hydroxide. When not in use, all solutions were stored at 4 °C in a refrigerator until HPLC analysis.

2.3. Sample preparation

Domestic sewage was sampled from drainage of the town of Qufu. Particulate matter was filtered onto a 0.45 μm nylon membrane filter (Alltech, Deerfiled, IL, USA), and the filtrate were adjusted to pH 2.5–3.0 with HCl. A SPE procedure for the isolation and preconcentration of the parabens from water was developed. Oasis HLB cartridges (3 mL, 60 mg), obtained from Waters (Milford, MA, USA), were installed on a vacuum manifold and preconditioned with 6 mL of methanol, and 6 mL of water. Thereafter, the aqueous samples (500 mL) were allowed to pass through the column at a rate of ~10 mL min^{-1}. The column was rinsed with 3 mL of a mixture of water and methanol (95:5, *v*/*v*). After the column was dried under vacuum for 10 min, the analytes were eluted with 10 mL of methanol. The eluate was evaporated to dryness under a gentle stream of nitrogen. The dry residue was redissolved in 1000 μL of acetonitrile, vortex mixed for 15 s, and transferred to an ampoule bottle.

2.4. Derivatization procedure

To a solution containing 50 μL of a standard parabens mixture in a 2-mL vial, 100 μL of derivatization reagent solutions and 150 μL of bicarbonate buffer were added. The vial was sealed and allowed to react in a water bath at 55 °C for 7 min. The derivatization procedure is shown in Fig. 1. After the reaction was completed, the mixture was taken to cool at room temperature, and 50 μL of 36% acetic acid were added to neutralize the mixture.

2.5. HPLC-ESI-MS

The separation of the selected pesticides was carried out using an HPLC system (consisting of vacuum degasser, autosampler and a binary pump) (Agilent Series 1290, Agilent Technologies, Santa Clara, CA, USA) equipped with Eclipce Plus C18 column (2.1 mm × 50 mm, 1.8 μm). Column temperature was maintained at 30 °C. The injected sample volume was 2 μL. Mobile phases A and B were 5% acetonitrile (containing 0.1% formic acid) and acetonitrile, respectively. The mobile phase composition initially was 50% A + 50%B, then changed to 5% A + 95% B by a linear gradient within 7 min. The flow-rate used was 0.2 mL min^{-1}. The