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Using water plug-assisted analyte focusing by micelle collapse in combination with microemulsion electrokinetic chromatography for analyzing phthalate esters

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ABSTRACT

Phthalate plasticizers are widely used in the plastics industry, but they have been detected in soft drinks, pharmaceuticals and food products. This study developed a method that uses water plug-assisted analyte focusing by micelle collapse and microemulsion electrokinetic chromatography (WPA-AFMC-MEEKC) for quantifying benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP), and diisodecyl phthalate (DIDP) in pediatric pharmaceuticals. The AFMC strategy was applied to improve the detection sensitivity, and a short water plug was introduced to assist micelle collapse in the micelle dilution zone for sample stacking. To carry neutral phthalates into the capillary through electrokinetic injection, sodium dodecyl sulfate (SDS) was added to the sample solution, and 8 mM SDS was selected as the optimal concentration. The optimized background solution (BGS) contained 16.13 mM phosphate buffer (pH = 2.5), 150 mM SDS, 0.75% *n*-octane (v/v), 5% 1-butanol (BuOH), 22.5% acetonitrile (ACN), and 15% isopropanol (IPA). Under the optimal separation conditions, four phthalates could be quantified within 20 min with enhancement factors of 58, 200, 86 and 90 for DIDP, DEHP, BBP, and DBP, respectively, compared to the conventional MEEKC mode. The limits of detection were within the range of 0.047–0.010 μg mL^{-1}. The accuracy of the method was within the range of 96–117%. The WPA-AFMC-MEEKC method was applied for the analysis of six pediatric pharmaceuticals, and the results demonstrated that the developed method is sensitive and accurate, allowing it to be used for quality control of pediatric pharmaceuticals.

1. Introduction

Phthalates are different ester forms of phthalic acid, and they have been widely used in the plastics industry to increase the flexibility, transparency, and longevity of plastics [1]. Because the phthalates are not covalently bonded to the plastics, they are easily released into the environment, particularly under certain conditions such as the introduction of heat. The toxicity of phthalates on the reproductive system may disrupt reproductive functions; these disruptions can include a decrease in anogenital distance, decreased serum estradiol levels, and prolonged estrous cycles, both in males and females [2–9]. Moreover, phthalates may also be responsible for carcinogenesis, including liver tumors, which may be associated with PPAR-α [10]. Therefore, the use of phthalates has been restricted in many countries, and monitoring the release of phthalates from food product and pharmaceutical containers is an important issue [1,11–13].

Many methods have been developed for measuring phthalates in various products, including high-performance liquid chromatography (HPLC) [14–18], gas chromatography coupled with mass spectrometry (GC–MS) [19–24], and LC coupled with tandem mass spectrometry (LC–MS/MS) [25,26]. Mass spectrometry methods provide the advantages of high selectivity and sensitivity, but the instrument costs are very high. Due to the high lipophilicity of phthalates, the column life span is greatly reduced when analyzing phthalates in complicated sample matrices when using extraction procedures for lipophilic compounds.

Green analytical chemistry (GAC) has gained great interest since 2000 which aims to make laboratory practice more environmentally friendly [27]. To minimize environmental burden caused by separation techniques, the twelve principles and three Rs (Reduce,

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Replace, and Recycle) are commonly mentioned in green analytical chemistry [28]. Gałuszka et al. later proposed the key components of green analysis include elimination or reduction of the use of chemical substances, minimization of energy consumption, proper management of analytical waste, and increased safety for the operator [29].

Capillary electrophoresis (CE) is a rapid analytical tool that has been developed in recent decades, and it is considered to be an environmentally friendly technique [27]. This technique has the advantages of fast separation speed and high theoretical plate number. In addition, the minimal sample (∼nL) and reagent consumption (∼μL), low column cost, and reduce the use of toxic solvent make CE become a favorable approach in considering the principles of GAC. The micellar electrokinetic chromatography (MEKC) mode of CE has been used to analyze phthalates [30–35]. However, the MEKC mode could not provide good performance for higher hydrophobic phthalates, such as diisodecyl phthalate (DIDP) [32]. Compared to MEKC, microemulsion electrokinetic chromatography (MEEKC) provides a better separation efficiency for highly hydrophobic compounds [36], and it has been widely used for analyzing pharmaceuticals with higher hydrophobicities [37,38]. MEEKC has also been applied for analyzing phthalates in different matrices, such as environmental soil and soft drinks [39,40].

Detection sensitivity is one of the main drawbacks of CE. To enhance the detection sensitivity, several on-line concentration methods have been developed for the CE system [41]. Because online concentration strategies could reduce the requirements in sample pre-treatment or sample derivatization for improving detection sensitivity, it added the advantages of CE in considering the principles of GAC. Stacking and sweeping are the most widely used on-line concentration techniques in CE. Stacking is a phenomenon in which sample ions accumulate at the boundary separating the low-conductivity sample plug and the high-conductivity background solution (BGS). In sweeping mode, a long sample plug without micelles is injected into a capillary pre-filled with BGS containing micelles. Sweeping occurs when micelles enter the sample zone and pick up and accumulate the analytes. Under sweeping conditions, the sample zone is devoid of micelles, and the organic solvent in the sample zone will decrease the sweeping efficiency. These requirements hinder the application of sweeping for the analysis of highly hydrophobic compounds such as phthalates.

Analyte focusing by micelle collapse (AFMC) is a relatively new online concentration technique that has shown good performance for neutral and hydrophobic compounds. In this technique, the sample zone contains micelles, and an additional micellar dilution zone (MDZ) is established between the sample zone and the BGS. The neutral analytes are carried by the micelles in the sample zone. The micelles collapse when they move into the MDZ, and the analytes are then stacked at the MDZ [42]. The successful application of AFMC combined with MEKC for the analysis of phthalates has been achieved by Quirino [43].

Contamination of pediatric pharmaceuticals with DIDP was reported in 2010 in Taiwan. Previous studies have proven that phthalates show toxicity to reproductive systems. The release of phthalates from the containers or tubes of pediatric pharmaceuticals should be highly concerning [8]. CE shows advantages in terms of being environmentally friendly and having a lower column cost. Although several CE methods have been developed for phthalate analysis, none of these methods included the reported pharmaceutical contaminated phthalate, DIDP, among their analytes probably due to the analytical difficulty. DIDP has the highest log P value (log P = 9.43) [44] among the commonly used phthalates, and the reported MEKC methods could not be used for the analysis of DIDP. In addition, contamination of pharmaceuticals with phthalates is generally in trace amounts, and it is important to use an online concentration strategy to ensure sensitive detection. To achieve both good sensitivity and high selectivity, this study developed a water plug-assisted analyte focusing by micelle collapse in combination with microemulsion electrokinetic chromatography (WPA-AFMC-MEEKC) mechanism for detecting phthalates in pediatric pharmaceuticals. Benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP), and diisodecyl phthalate (DIDP) are the most commonly detected phthalates in pharmaceutical products, and they were all included as our analytes. The optimized WPA-AFMC-MEEKC method was validated according to the ICH guidelines. Finally, the developed method was applied to quantify these phthalates in six pediatric pharmaceuticals obtained from our local hospital to demonstrate its applicability.

2. Experimental

2.1. Chemicals

Benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP), isotope DEHP-d4 and diisodecyl phthalate (DIDP) standards and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH), phosphoric acid (>85%), 1-butanol (BuOH), and *n*-octane were purchased from Merck (Darmstadt, Germany). Acetonitrile (ACN) and methanol (MeOH) were purchased from Mallinckrodt (Paris, KY, USA). Isopropyl alcohol (IPA) was purchased from Scharlau (Barcelona, Spain). All reagents and solvents used were of analytical or chromatographic grade. All of the neutral phthalate stock solutions were prepared in IPA with a concentration of 1 mg mL^{-1} and were stored at 4 °C. The pediatric pharmaceuticals were purchased from the National Taiwan University Hospital, Department of Pharmacy.

2.2. Instrumentation

All CE experiments were performed on a P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA, USA) equipped with a photodiode array detector. The CE instrument was controlled by 32-Karat software 7.0 (Beckman Coulter, Fullerton, CA, USA). A fused-silica capillary from Polymicro Technologies (Phoenix, AZ, USA) was used for the separation.

All CE procedures were performed on a 55 cm column (45 cm effective length) with a 50 μm I.D. fused-silica capillary. The UV detector was set at 214 nm. The separation was performed at 25 °C, and the applied voltage was set at −22 kV. The new capillary was conditioned by flushing sequentially with 1.0 M NaOH for 30 min, 0.2 M NaOH for 30 min, and deionized water for 30 min. At the beginning of each run, the capillary was washed with 0.2 M NaOH for 5 min, MeOH for 5 min, deionized water for 5 min, and BGS for 5 min. The capillary was washed using the same procedures excluding BGS at the end of each day.

2.3. Experimental procedures

2.3.1. WPA-AFMC-MEEKC method

The background solution (BGS), which contained 16.13 mM phosphate buffer, 150 mM SDS, 0.75% *n*-octane (v/v), 5% BuOH, 22.5% ACN, and 15% IPA, was prepared fresh daily followed by 30 min of sonication. The sample stock solution was diluted in the sample matrix (SM), which consisted of 8 mM SDS in 25 mM phosphate buffer. Prior to sample injection, deionized water was injected into the capillary at 0.5 psi for 30 s. The sample was injected into the capillary at 10 kV for 60 s.