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Simultaneous determination of polyvinylchloride plasticizers di(2-ethylhexyl) phthalate and tri(2-ethylhexyl) trimellitate and its degradation products in blood by liquid chromatography-tandem mass spectrometry

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ABSTRACT

Di(2-ethylhexyl) phthalate (DEHP) and tri(2-ethylhexyl) trimellitate (TEHTM or TOTM) are common plasticizers that are also largely used for PVC medical devices, e.g. bags and tubing for blood transfusions and infusions. The leachability of medical devices is a well-known situation of increasing toxicological concern. To assess the migration of plasticizers from PVC medical devices into human blood we developed and validated an analytical method for the determination of DEHP and TOTM in combination with the determination of their primary degradation products mono(2-ethylhexyl) phthalate (MEHP), 1,2-di(2-ethylhexyl) trimellitate (1,2-DEHTM) and 2-mono(2-ethylhexyl) trimellitate (2-MEHTM). The presented method involves liquid–liquid extraction of the analytes from the blood followed by the subsequent analytical separation and detection using LC–MS/MS analysis. The validation of the procedure showed a good precision in the range of 1.8 to 5.3%. Mean accuracy ranged from 86% for 1,2-DEHTM to 109% for MEHP. LOQ was found to be 2 to 5 μg/L for each of the analytes. Additionally, the method is characterised by its wide linear range up to 2 mg/L each for the degradation products of TOTM to 100 mg/L for the parent plasticizer DEHP. The presented method promises to be of major advantage for further studies as it allows for the first time the simultaneous determination of DEHP and TOTM in human blood in combination with the analysis of their degradation products that render possible to investigate the leachability of a broad range of PVC medical devices in human blood using only one analytical method.

1. Introduction

Polyvinyl chloride (PVC) is the third-most widely produced polymer and holds the largest share of polymer based medical products. PVC is often used due to its inertness and high transparency and because it is rather inexpensive and extremely versatile. PVC components are often used in medical devices. Typical applications comprise bags and tubing for blood transfusions, infusions and dialysis systems [1,2]. To induce and maintain processability and flexibility technical PVC contains additives, such as plasticizers. The plasticizer concentration in PVC medical devices often ranges between 20 and 40% of the total weight formulation [3,4]. The plasticizers are not chemically bound to PVC and can thus migrate through direct contact from medical products into blood, infusion liquids, and other contents. The leachability into blood is considered to be increased due to its lipophilicity.

The most commonly used plasticizer for PVC products is still di(2-ethylhexyl) phthalate (DEHP) [4–6]. But, severe toxicological concerns exist regarding adverse developmental and reproductive effects of DEHP [6–8]. Additionally, due to sufficient evidence in experimental animals, the IARC classified DEHP as possibly carcinogenic to humans (Group 2B) [4]. Therefore, the EU has established that DEHP shall no longer be used in toys or childcare articles [9]. In medical products, however, DEHP is still in use.

It is known that DEHP migrates from PVC into blood products and thus into patients undergoing transfusions or maintenance haemodialysis [10–12]. The leachability into blood may lead to a significant exposure to DEHP [7,8], especially for 'high-risk-patients', as pregnant women, male neonates and long-term transfusion patients [4,6]. Apart from DEHP, also its degradation product mono(2-ethylhexyl) phthalate (MEHP) was found in blood products [11,12] and enteral nutrition products [13]. In vivo, DEHP

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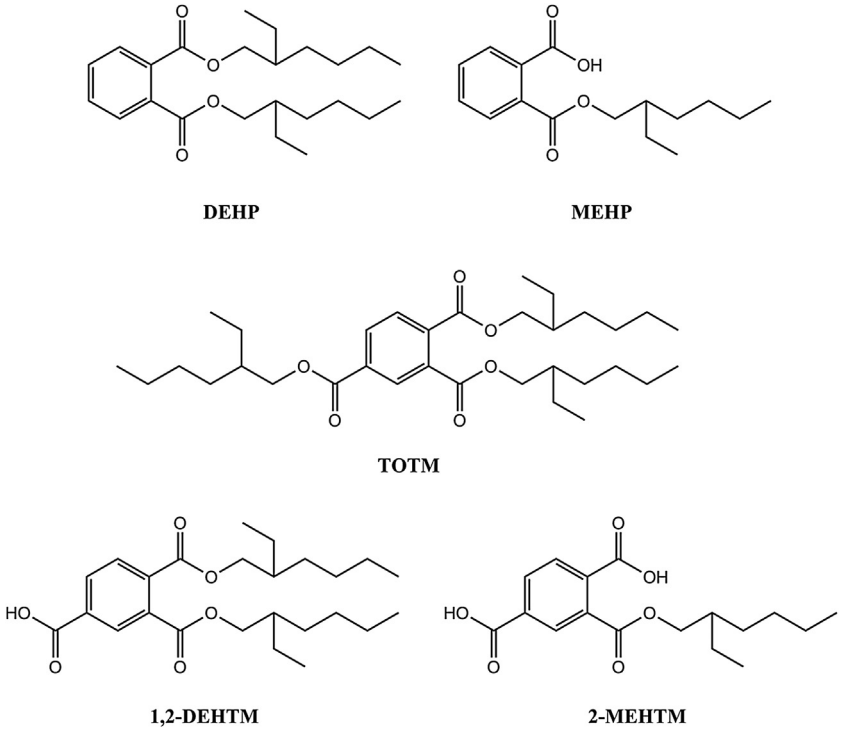

Fig. 1. Chemical structures of the five analytes DEHP, MEHP, TOTM, 1,2-DEHTM and 2-MEHTM.

is rapidly metabolised to the monoester MEHP and further oxidative metabolites [14]. Both, DEHP and MEHP contribute to DEHP toxicity [4,6]. It is argued that DEHP is likewise hydrolysed in blood products by esterase activities [15], or that the sterilization process of PVC medical products may lead to the formation of MEHP [13]. The levels of exposure to DEHP and MEHP released from medical devices are highly variable (ranging from μg per kg body weight to several grams per year for patients with chronic treatments) and have not been extensively studied (reviewed by [3,6,16]).

Since DEHP toxicity is an increasing matter of concern, there is a growing demand for alternative plasticizers [2,17], like tri(2-ethylhexyl) trimellitate (TEHTM or TOTM). First results indicate that TOTM actually possesses a lower toxicity than DEHP [2,18–20]. Moreover, a higher chemical stability can be assumed indicated by a significantly decreased leachability of TOTM from PVC medical devices into intravenous preparations [21] and into blood [10]. However, up to now there is still little data on TOTM exposure, toxicity and leachability from medical devices. There is an urgent need for further studies to clarify if TOTM really is a superior alternative to DEHP. With regard to TOTM toxicity, the question arises as to whether TOTM shows similar characteristics as DEHP and is hydrolysed in PVC or in blood products to the corresponding metabolites. Based on the similar structure to DEHP, it can be assumed that TOTM is hydrolysed to di(2-ethylhexyl) trimellitates (DEHTM) and mono(2-ethylhexyl) trimellitates (MEHTM) (cf. Fig. 1).

A major limitation for further studies including a reliable risk assessment is, that there are very few suitable analytical methods for the determination of parent plasticizers in blood (reviewed by Bernard et al. [3]). Existing methods usually focus on the migration of parent plasticizers into synthetic oral fluids [22] and the determination of DEHP metabolites in body fluids after human metabolism [8]. However, to assess the actual exposure to plasticizers from medical devices it is necessary to determine both, the parent plasticizer and their primary degradation products in blood. Most methods enable the determination of either TOTM or DEHP in blood using liquid–liquid extraction followed by HPLC-UV analysis [10,11,23,24]. One method employs GC-MS analysis of TOTM as trimellitic acid after derivatisation [25]. However, these methods are mostly characterised by relatively high limits of quantitation due to the used unspecific detection technique. Furthermore, there are methods for the simultaneous determination of DEHP and MEHP in blood using LC–MS/MS analysis after liquid–liquid extraction [26], or online-SPE LC–MS analysis [12]. The working group of Ito et al. developed LC–MS/MS methods for the determination of TOTM [21] and for the simultaneous analysis of DEHP and MEHP [27], however, not for blood samples but drugs and simulants. To our knowledge the occurrence of hydrolysis products of TOTM in blood or other liquids in contact with PVC medical devices has not yet been studied.

The objective of our work was therefore to develop a reliable and straightforward LC–MS/MS method for the determination of DEHP and TOTM in human blood with simultaneous analysis of the degradation products of each plasticizer. Accordingly, the method included the following analytes shown in Fig. 1: (1) DEHP, (2) its primary metabolite MEHP, (3) TOTM and its potential metabolites (4) 1,2-di(2-ethylhexyl) trimellitate (1,2-DEHTM) and (5) 2-mono(2-ethylhexyl) trimellitate (2-MEHTM) that were selected as representatives of the potential diester and monoester metabolites of TOTM, respectively.

2. Materials and methods

2.1. Reagents and chemicals

Di(2-ethylhexyl) phthalate (DEHP) was purchased from Fluka. Tri(2-ethylhexyl) trimellitate (TOTM) and 3,4,5,6-D_4-di(2-ethylhexyl) phthalate (D_4-DEHP) were purchased from Sigma Aldrich (Steinheim, Germany). Mono(2-ethylhexyl) phthalate (MEHP) and 3,4,5,6-D_4-mono(2-ethylhexyl) phthalate (D_4-MEHP) were synthesised by the Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine of the University of Erlangen-Nuremberg as described elsewhere [28] with a stated chemical purity of >95%. 2-Mono(2-ethylhexyl) trimellitate (2-MEHTM) and 1,2-di(2-ethylhexyl) trimellitate (1,2-DEHTM) were