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How to solve the problem of co-elution between two compounds in liquid chromatography through the first UV derivative spectrum. A trial on alternative plasticizers to di(2-ethylhexyl) phthalate

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ABSTRACT

To meet new regulations, alternative plasticizers to di(2-ethylhexyl) phthalate (DEHP) are now commonly used in the manufacturing of medical devices. These are: acetyl tri-n-butyl citrate (ATBC), bis (2-ethylhexyl)adipate (DEHA), dioctyl terephtalate (DEHT), di-isononylphtalate (DINP), diisononylcyclohexane-1.2-dicarboxylate (DINCH) and trioctyltrimellilate (TOTM). An HPLC-UV analysis was previously developed to characterize four of them. However, two compounds were systematically co-eluated: DEHP with DEHA and DEHT with DINP. The first derivative of UV spectra and photodiode array detection allow the quantification of DEHA and DINP. Moreover, for each plasticizer, maximum wavelength absorbance was chosen to be as specific as possible. Quantification ranged from 0.3 to 750 µg/mL according to the plasticizer. The assays were validated by analysis of variance. Our method was validated by determining the following parameters: specificity, linearity, limits of detection and quantification. The relative biases were inferior to 5% for ATBC, DEHP, DEHA and DINCH and inferior to 10% for DEHT, DINP and TOTM. Plasticizers were extracted with tetrahydrofuran and methanol. The developed method was then used to determine the composition of plasticizers in several medical devices used in clinical service. The major plasticizers were quantified from 19% to 40% w/w, traces of DEHT were found in six medical devices and DEHP in five.

1. Introduction

Polyvinyl chloride (PVC) is one of the most common plastics used nowadays, especially in medical devices (MDs) (e.g. infusion tubing, dialysis sets, endotracheal tubes, feeding tubes...) [1]. Indeed, it presents good physicochemical properties: inertness, chemical stability, biocompatibility, clarity, high transparency, durability, chemical and mechanical resistance, easy sterilization and is cost-friendly. Plasticizers are used to improve the softness and flexibility of PVC products and can attain nearly 40% (w/w) of the weight of the plastic [2].

Historically, di(2-ethylhexyl) phthalate (DEHP) was the most widely used plasticizer because of its properties. But it is not strongly bound to the matrix polymer in flexible PVC and can migrate from the MD into the medication. The presence and release of DEHP have therefore been evaluated in different MDs [3]. In 2008 [4] and more recently in 2015 [5] the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) highlighted situations at high risk of exposure to DEHP and analyzed data concerning alternative plasticizers to DEHP. They concluded it was essential to evaluate exposure to these compounds in the medical context.

According to the European Directive 2007/47/EC [6], MDs must fulfil new essential requirements. MDs containing phthalates classified as Carcinogenic, Mutagenic and toxic for Reproduction (CMR 1a or 1b) must be clearly labelled as devices containing phthalates. The manufacturer must provide a specific justification for the use of such substances if the device is intended for treatment on patients at risk, as defined in the SCENIHR report. In France, the use of DEHP has

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been restricted in MDs for paediatric, neonatology and maternity care from July 1, 2015 [7,8].

These new regulatory guidelines have obliged MD manufacturers to use alternative plasticizers to DEHP [2]. The preferred six are: acetyl tri-n-butyl citrate (ATBC), bis (2-ethylhexyl) adipate (DEHA), dioctyl terephthalate (DEHT), di-isononylphthalate (DINP), diisononylcyclohexane-1.2-dicarboxylate (DINCH) and trioctyltrimellitate (TOTM). However, to assess their toxicity vis-à-vis humans, their ability to migrate [9] and the risk of patient exposure, it was necessary to develop analytical methods and a migration model.

Some analytical methods have been designed to analyze plasticizers in infused solutions, biological liquid or the environment [10] and an in vitro migration model [9,11] was conceived for alternative plasticizers in PVC MDs to measure the amount of TOTM released from extension sets used in adult intensive care units (ICU).

In a recent literature review, Bernard et al. [12] have indicated several analytical methods developed to identify and quantify DEHP alternative plasticizers in MDs or human body fluids. These can be divided into two types, direct or indirect methods, according to the need for a preliminary step to extract the plasticizer from the PVC. Vaccher et al. [13] conceived a method using a porous graphitic carbon (PGC) stationary phase in supercritical fluid chromatography (SFC) with an ELSD. This method was capable of detecting four alternative plasticizers (ATBC, DEHA, TOTM and DEHT). Among indirect methods, the most sensitive and specific methods used are gas chromatography (GC) and liquid chromatography (LC). LC can be combined with evaporative light scattering detection (ELSD) [14] to detect four alternative plasticizers: ATBC, DEHA, DEHT and TOTM. Both GC and LC can be coupled with mass spectroscopy GC–MS [15,16] and LC-MS [17]. Detection by mass spectrometry is not widely accessible because of its cost. Radaniel *et al.* developed an LC-UV method to detect and quantify five plasticizers (ATBC, DEHP, DEHT, DINCH and TOTM) in the same analysis [18]. In their method, benzylbutylphtalate (BBP) was chosen as internal standard (IS) because, being prohibited, it could not be found in MD. However, they were unable to quantify DEHA and DINP because the compounds were systematically co-eluted with DEHP and DEHT, respectively.

The aim of this study was to develop an LC-UV method based on improving Radaniel's assay [18] using a derivative spectrum chromatogram method to simultaneously quantify the six alternative plasticizers as well as DEHP.

2. Material and methods

2.1. Chemicals and consumables

2.1.1. Plasticizers

DEHP and six alternative plasticizers were studied: ATBC, DEHA, DEHT, DINCH, DINP and TOTM. BBP, prohibited in MDs, was chosen as IS. All plasticizers were obtained from Sigma Aldrich (Saint Quentin Fallavier, France) except for the Hexamoll® DINCH®, which was offered by the BASF SE Company (Ludwigshafen, Germany).

2.1.2. Solvents

Acetonitrile (ACN) (HiPerSolv Chromanorm®), Methanol (HiPerSolv Chromanorm®) and Tetrahydrofuran (THF) (HiPerSolv Chromanorm®) were purchased from VWR (Fontenay-sous-Bois, France). All solvents were HPLC gradient grade. Technical methanol, used for washing, was bought from Brabant (Tressin, France). Ultrapure water was obtained from a Purelab classic Elga water system (Veolia, Wasquehal, France).

2.1.3. Laboratory consumables

Flasks and beakers were purchased from VWR and plasticizer-free plastic consumables such as tips for micropipettes (Biohit, Helsinki, Finland), conic Eppendorf® plastic tubes (Eppendorf®, Fontenay-sous-Bois, France) were used for the experiments. Glassware was mainly used to avoid cross-contamination with plasticizers contained in many plastic materials.

2.2. Preparation of stock and working solutions

Two stock solutions were prepared for each plasticizer by diluting a quantity accurately weighed on a precision scale (model 220 XT, precision ± 0.1 mg, series 220 XT, Precisa Gravimetrics AG, Dietikon, Switzerland) in ACN. The concentrations of the stock solutions of BBP, DEHP, DEHT, DINP and TOTM were 0.5 mg/mL and 10 mg/mL for ATBC, DEHA and DINCH. The first solution served for calibration standards (CS) and the second for validation standards (VS). Plasticizers were directly weighed in volumetric flasks provided with a glass stopper to avoid diluent evaporation. Each work solution was prepared in a volumetric flask by diluting the correct volume of corresponding stock solution with ACN. The concentrations of stock and work solutions were calculated as functions of their ability to absorb in UV after studying each UV spectrum of the plasticizers [14].

2.3. Instrumental analysis

The analytical method developed by Radaniel [18] with an Ultra-High-Performance Liquid Chromatographic (UPLC) system (Shimadzu®, Noisiel, France) was used. This system was equipped with a degassing DGU-20A3R unit to eliminate gases in mobile phases, two LC-20ADXR solvent delivery units (Prominence UFLCXR series), a SIL-20ACXR autosampler, a CTO-20AC column oven and an SPD-M20A photodiode array detector.

The mobile phase was composed of ACN and water. During the run, the percentage of ACN was evolved. It started from 65% during 3.75 min, then increased to 83% until 6.25 min and finally raised to 95% until 11 min before a return to elution conditions of the beginning. The flow-rate was fixed at 2 mL/min and each run lasted 13 min. Oven temperature was maintained at 35 °C. Separation of plasticizers was carried out on a Kinetex™ C8 column (100×4.6 mm i.d., 2.6 µm) (Phenomenex®, Le Pecq-France). Spectra were recorded from 200 to 300 nm.

2.4. Derivative spectrum chromatographic method

To elucidate the co-elution between DEHP and DEHA (retention time (RT) 6.2 min) and between DEHT and DINP (RT 7.5 min), it was decided to identify and quantify these two plasticizers using the UV first derivation spectrum to differentiate the co-eluted compounds. Diode array detection (DAD) and the use of a specific module (i-PDeA™) of Shimadzu® Labsolution software [19] permitted the first UV derivation spectrum of each plasticizer. The use of the derivative spectrum chromatographic method has already been published [20–24]. DAD and a specific software module make it possible to detect and quantify each plasticizer at its specific wavelength (corresponding to maximum specific absorbance) and provide selectivity and sensitivity in analysis. An intelligent peak deconvolution analysis (i-PDeA™) derivative spectrum chromatogram method was developed by Shimadzu® as a new data processing technique for HPLC-DAD. A derivative spectrum is created through differential processing of the UV–visible absorption spectrum at each measurement time. Plotting derivative spectrum values at the specified wavelength against RT creates a derivative spectrum chromatogram that is able to separate co-eluted peaks. The high selectivity of the derivative spectrum chromatogram can detect unexpected impurities and quantify the target component only, without interference from components that elute simultaneously. This method can separate and quantify two chromatographically co-eluted peaks in a data set established for a mixture of two components [19].