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Fast Phthalate Detection in Textile Samples: A LC-MS/MS Screening Method Using Precursor Ion Scans †

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Abstract: Phthalate esters (PAEs), commonly known as phthalates, are widely used as plasticizers in the textile industry to improve the flexibility, transparency, and durability of various materials. However, the potential adverse health effects associated with them (such as endocrine disruption, reproductive toxicity, and possible carcinogenicity) require strict control measures. In this work, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to determine phthalates in textile samples. By using precursor ion scans (specifically the precursor ion 149, characteristic of phthalates), the procedure improves the specificity of the analysis. The developed method was successfully applied to different textile samples, and the results obtained were comparable to those from the official gas chromatography method (GC-MS/MS). The proposed method enables the rapid and effective detection of phthalates in quality control laboratories within the textile industry.

Keywords: LC-MS/MS; phthalates; precursor ion mode; quality control; textile industry

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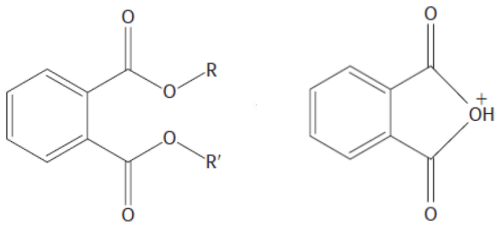
1. Introduction

Phthalate esters (PAEs) are ortho-di-esters of phthalic acid with a distinctive chemical structure involving two ester groups attached to the ortho positions of a benzene ring. This generic structure (see Figure 1a) can be modified by varying the alcohol radicals R and R′, resulting in numerous phthalate derivatives with diverse properties [1].



(**a**) (**b**)

Figure 1. (**a**) General structure of ortho-phthalate esters (PAEs). (**b**) Structure of fragmentation ion of PAEs at *m*/*z* 149.

Phthalates are widely used as plasticizers in various PVC-based materials in the textile industry to enhance their flexibility, transparency, and durability. PVC is a naturally rigid polymer, made flexible by the addition of phthalates, making it suitable for a range

of textile applications, including clothing [2,3]. As a result, PAEs are present in many plastic-based consumer and industrial products, and because phthalates are not chemically bound to plastic polymers, they are widely released and dispersed into the environment.

Exposure to PAEs has been associated with negative effects on human health. Specifically, PAEs are considered endocrine disruptors, and some are classified as possible carcinogens [4,5]. Dermal exposure, particularly through contact with textiles containing phthalates, is an important exposure route, especially for children, who are particularly vulnerable to these harmful effects [6]. Due to these adverse effects, most countries legislation has imposed restrictions on the use of phthalates in various products and clothing (primarily those intended for children) and has defined mechanisms for their control [1].

Biomonitoring phthalates in plastic-based products in general, and textile products in particular, is an important task to protect consumer health. For this task, analytical technologies (such as GC-MS) enable the simultaneous determination of different regulated phthalates in a single, rapid analysis. Additionally, over the past decade, different phthalates have been used to replace the currently regulated compounds (simply modifying the R and R′ radicals) [3]. Therefore, it is important to study the presence and exposure to these other PAEs, as the substitute phthalates are likely to have similar toxicities to those that are prohibited. Thus, it is crucial to develop methodologies capable of detecting and quantifying any phthalate in a single, rapid analysis, regardless of its R and R′ radicals.

Recent developments have introduced novel non-targeted screening methods using liquid chromatography coupled with mass spectrometry for this purpose. In these methods, precursor ion scans are a type of scan mode in tandem mass spectrometry (MS/MS), where the instrument selects precursor ions (the ions produced from the analyte after ionization) and monitors for specific product ions resulting from fragmentation. In the case of phthalate esters, the most intense peak in their electron ionization mass spectrum typically appears at *m*/*z* 149, due to the rapid formation and stability of a particular ion, as illustrated in Figure 1b. The only exception is dimethyl phthalate, where both R groups are methyl (R = R′ = -CH_3), and the base peak appears at *m*/*z* 163 [7,8].

Yinon in 1988 [9] studied the mass spectral fragmentation pathways of phthalate esters with alkyl side chains. These PAEs generally fragment in two ways: first, through a McLafferty rearrangement, involving the transfer of two hydrogen atoms and the loss of $[R\text{-}2H]^-$; and second, through α-cleavage of the molecular ion, leading to the loss of $[OR]^-$. From there, several possible routes can produce protonated phthalic anhydride, detected at *m*/*z* 149 (as explained by Yin et al. in 2014 [10]). In the case of dimethyl phthalate, the fragmentation is different. Its breakdown is simpler: a methoxyl group is removed via α-cleavage, resulting in a base peak at *m*/*z* 163. With collision activation, the ion at *m*/*z* 163 loses another methoxyl group, producing a peak at *m*/*z* 133, which continues to fragment further.

The development and implementation of such advanced analytical techniques are essential for effectively monitoring phthalate levels, ensuring compliance with safety regulations, and protecting public health. By focusing on the characteristic *m*/*z* 149 and 163 ions, LC-MS/MS provides a robust tool for the rapid and precise detection of phthalates in various samples, including textiles and other consumer goods. This capability enhances regulatory compliance and helps mitigate potential health risks associated with phthalate exposure.

In the present work, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the screening of any phthalate present in textile samples. Using precursor ion scans (specifically the 149 and 163 *m*/*z* precursor ions characteristic of phthalates), the procedure ensures the detection of any PAE. The developed method was successfully applied to different textile samples, and the results obtained were comparable to those from the official gas chromatography method (GC-MS/MS). The proposed LC-MS/MS method provides a useful tool for the rapid detection of phthalates in various types of samples, especially from the textile industry.

2. Material and Methods

2.1. Apparatus and Reagents

The liquid chromatography-tandem mass spectrometry method was developed using a Shimadzu HPLC-LC-20ADXR system (Shimadzu Corporation, Kyoto, Japan) coupled with a SCIEX Triple Quadrupole 4500 system (SCIEX, Framingham, MA, USA). A CTC PAL HTC-xt with a DLW autosampler (CTC Analytics, Zwingen, Switzerland) was used for sample injection. Analyst software (SCIEX) version 1.6.3 HF4 was used for data acquisition, and LC-MS/MS data were processed using MultiQuant software (SCIEX) version 3.0.3.

For comparative analysis, a gas chromatographic analysis was performed using an Agilent 7890B gas chromatograph coupled to a 7000 C Series Triple Quad Mass-Spectrometer detector (GC-MS/MS) from Agilent Technologies (Agilent, Santa Clara, CA, USA).

Twenty phthalates were considered in this work to verify the reliability of the proposed screening method: benzyl butyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), butyl octyl phthalate (BOP), dibutyl phthalate (DBP), dicyclohexyl phthalate (DCHP), diethyl phthalate (DEP), dihexyl phthalate (DHP), diisobutyl phthalate (DIBP), diisodecyl phthalate (DIDP), diisoheptyl phthalate (DIHP), diisononyl phthalate (DINP), diisooctyl phthalate (DIOP), diisopentyl phthalate (DIPP), dimethyl phthalate (DMP), dinonyl phthalate (DNP), dioctyl phthalate (DNOP), dipentyl phthalate (DNPP), dipropyl phthalate (DPRP), diundecyl phthalate (DUP), and isopentyl pentyl phthalate (PIPP). Individual standards for phthalate esters were obtained from LGC Group (Middlesex, UK), Sigma-Aldrich (Madrid, Spain), and Scharlab (Barcelona, Spain). A commercial isomer standard mixture was used for DIPP, PIPP, and DNPP from LGC Group. Benzyl benzoate (BB), obtained from Scharlab, and deuterated butyl benzyl phthalate (BBP-d4) and deuterated bis-(2-ethylhexyl) phthalate (DEHP-d4), both supplied by Laboratorios CIFGA S.A. (Lugo, Spain), were used as internal standards. Stock solutions of 1000 mg/L for each phthalate were individually prepared in a solvent mixture of tetrahydrofuran:acetonitrile (1:2). Working solutions of 100 mg/L, 10 mg/L, and 1 mg/L were prepared from the stock solutions.

All solvents used for chromatography were obtained from Merck (Darmstadt, Germany): tetrahydrofuran, hexane, acetonitrile, and methanol. Ammonium acetate, used for preparing the mobile phase, was also from Merck. Ultra-pure water was produced using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Samples and Sample Pretreatment

The ten textile samples analyzed in this work consisted of various materials and garment components (both finished products and raw materials) and included buttons, multicolor positional printing pastes, appliqués, plastic fabrics, sequins, handles and belts.

All of these samples were phthalate-positive and were obtained from a quality control laboratory accredited for monitoring and verifying compliance with legal phthalate content. This study also included four samples from interlaboratory tests for the determination of phthalates: two liquid samples of chemicals used in the textile industry and two samples of textile raw materials.

To compare the developed method with the official methodology for determining phthalates, all textile samples were analyzed using both methods. Samples were extracted following the procedure described in a previous paper [3], using acetonitrile instead of hexane for the re-precipitation of any PVC polymer, and the extracts were analyzed according to both the official test method from the United States Consumer Product Safety Commission (US-CPSC) [11] and the method developed in this work.

3. Results and Discussion

After different steps for optimizing the LC-MS/MS method, the optimal results were summarized as follows.

3.1. Liquid Chromatography Experimental Conditions

HPLC separation was performed using a Zorbax Eclipse XDB-C18 column (150 × 2.1 mm, 5 μm). Mobile phase A and mobile phase B were 10 mM ammonium acetate and acetonitrile, respectively. The gradient program used, along with other chromatographic conditions, is presented in Table 1.

Table 1. Optimized LC-MS/MS conditions

LC Conditions							
Column:	Zorbax Eclipse XDB-C18 150 × 2.1 mm, 5 μm						
Column temperature:	25 °C						
Injection volume:	5 μL						
Autosampler temperature:	8 °C						
Mobile phases:	A: 10 mM ammonium acetate; B: acetonitrile						
Run time:	14 min						
Flow rate:	0.4 mL min^{-1}						
Gradient:	Time (min)	0.0	0.5	5.0	11.0	11.1	14.0
	%B	80	80	99	99	80	80
MS/MS Conditions							
Scan type:	Precursor Ion (Pre)						
Scan rate:	1000 Da s^{-1}						
Polarity:	Positive						
Precursor of:	149 and 163 Da (two experiments)						
Scan Q1 range:	150 to 800 Da						
Source/Gas parameters							
Ion source:	Turbo Spray						
Curtain gas (CUR):	35 psi						
Collision gas (CAD):	9						
Ion Spray Voltage (IS):	4500 V						
Temperature (TEM):	300 °C						
Ion source Gas 1 (GS1):	70 psi						
Ion source Gas 2 (GS2):	50 psi						
Compound parameters							
Declustering Potencial (DP):	40 V						
Entrance Potencial (EP):	10 V						
Collision Energy (CE):	25 V						
Collision Cell Exit Potencial (CXP):	10 V						

The rinsing solutions for the autosampler, both before and after aspiration, were as follows: solvent 1 was methanol:water (90:10, *v*/*v*), and solvent 2 was methanol:water (80:20, *v*/*v*). The rinsing sequence consisted of two washes with solvent 1 and two washes with solvent 2, using a filling rate of 5 μL/s and one filling stroke. The injection rate was 25 μL/s, with a pre- and post-injection delay of 0.5 s.

3.2. Mass Spectrometry Experimental Conditions

The SCIEX Triple Quadrupole 4500 was operated in precursor ion mode. The Turbo V source was used with an Electrospray Ionization (ESI) probe in positive polarity, and parameters were optimized for maximum sensitivity.

In precursor ion scanning, the first quadrupole (Q1) was set to scan the target mass range, while quadrupole Q2 functioned as the collision cell, and the third quadrupole (Q3) was set to transmit ions with fixed *m*/*z* values (149 and 163). The detector recorded all

precursor ions in Q1 that generated a fragment with the specified *m*/*z* in Q3. This method enabled the selective detection of precursor ions that produce a specific, constant product ion after ESI. Two selective precursor ions were monitored for all target analytes. The optimal conditions achieved are summarized in Table 1.

3.3. Application

Under the conditions described above, the chromatograms of two sets of phthalate standards (at 0.5 μg mL^{-1} level) are presented in Figure 2. All PAEs were detected using the precursor ion scan at *m*/*z* 149, with the expected exception of the dimethyl phthalate peak, which appears at *m*/*z* 163, as previously mentioned.

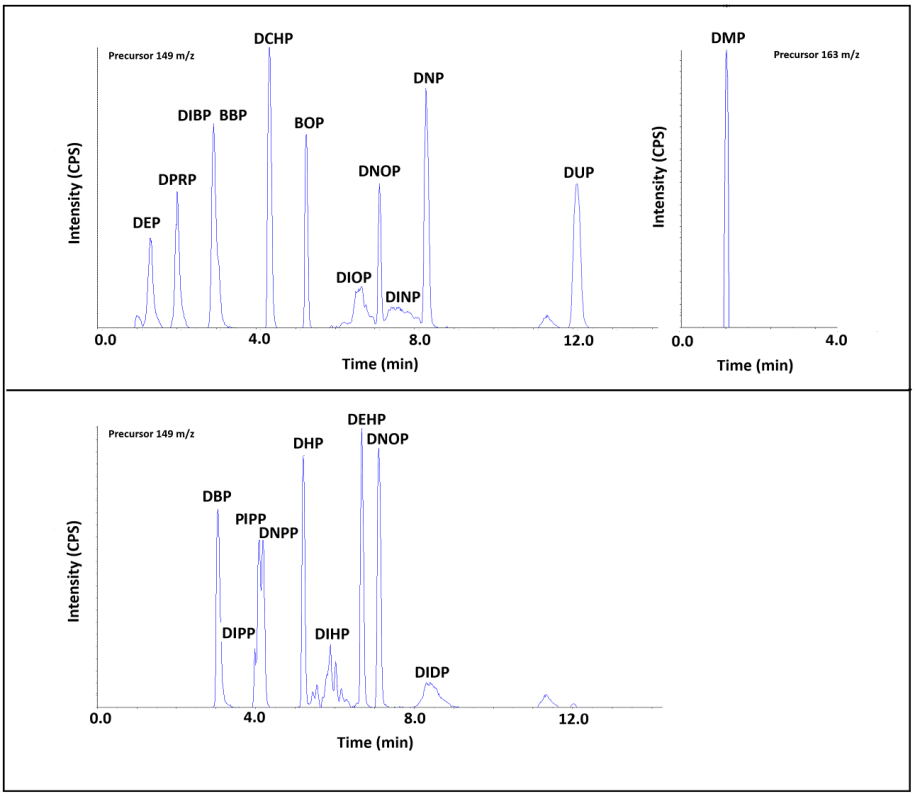


Figure 2. Chromatograms of two sets of PAE standards (0.5 μg mL^{-1}).

Textile samples were analyzed using both the developed method and the official method, yielding comparable results. Figure 3 displays the chromatogram obtained for a textile sample (the plastic handle of a handbag), which was found to be positive for four phthalates (DBP, DEHP, DINP, and DIDP) by the official method.

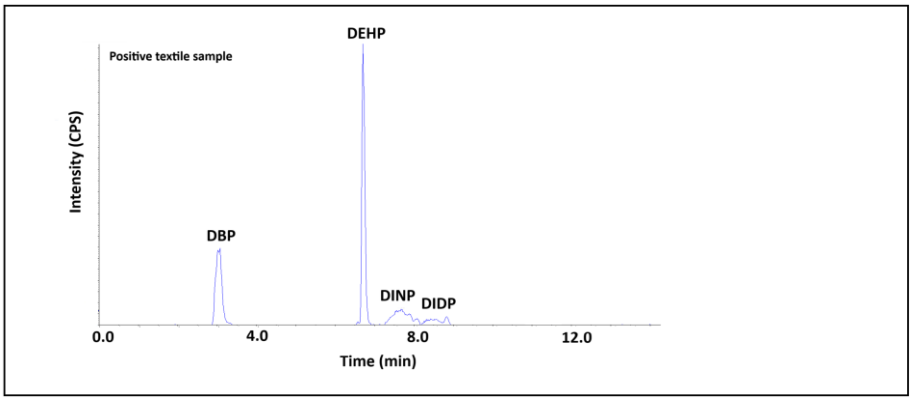


Figure 3. Chromatogram of a PAE-positive textile sample.

As shown, this new method is capable of detecting any PAE present in the sample. In fact, a peak from an unknown PAE (possibly an impurity in the standards mixture) appeared at the end of the chromatogram (11.3 min).

In conclusion, the developed LC-MS/MS method is an appropriate alternative for screening PAEs in textile samples and is possibly applicable to other sectors.

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