Development of a reliable extraction method for the identification and quantification of 7 phthalate esters in recycled paperboard materials intended for food contact applications.

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Phthalates are associated with various health concerns, and therefore guidelines are enforced by national and international restrictions worldwide in order to curb their use. They are present in hundreds of products, such as vinyl flooring, medical tubing, personal-care products, and even plastic and paperboard packaging. People are exposed to these chemicals by eating and drinking foodstuffs that have come in contact with materials containing phthalates. So far, studies on determining such contaminants in food packaging have focused mainly on monomers and a few additives used in high concentrations (Lim, 2015; Lithner et al., 2011; Wiesinger et al., 2021). This study attempts to develop a fast, simple, and reliable extraction method for the identification and quantification at very low concentrations of this type of chemicals in recycled paperboard materials intended for food contact applications. To this end, three extraction methods were examined and evaluated for their effectiveness and accuracy in isolating phthalate esters in recycled paperboard samples: the Soxtec Extraction (SE), the Ultrasound-Assisted Extraction (UAE), and the Head Space Solid Phase Micro Extraction (HS-SPME). The phthalate ester mix used for this study consisted of the 7 most commonly found components: Di-n-butyl-phthalate (DBP), Butyl benzyl phthalate (BBP), Di-2-ethyl hexyl phthalate (DEHP), Di-n-octyl phthalate (DOP), Dimethyl phthalate (DMP), Diethyl phthalate (DEP), and Di-2-ethyl hexyl adipate (DEHA). Following the extractions, a Gas Chromatography-Mass Spectrometry (GC/MS) analysis was performed for the determination of the phthalate esters. Additionally, the paperboard samples were spiked with the phthalate ester mix and then subjected to migration experiments using Tenax as a dry-food simulant.

Four different types of paperboards, commercially available in the Greek market as food-packaging materials, were used in the study, 3 from 100% recycled pulp matter (R1, R2, & R3) and 1 virgin material (V). The aforementioned phthalate ester mix was used as the standard solution for the contamination of the paperboards.

The extraction conditions were optimized for all methods tested by comparing the relative recovery rates resulting from the integration of the contaminant peaks in the respective chromatograms (yields) to the compound exhibiting the highest recovery. The identification of the compounds was achieved using the Wiley 7, NIST 2005 mass spectral library and was further supported by the comparison of their linear retention indices (Kovats indices) to those of the reference standards and/or published data (NIST, 2005; Van den Dool & Dec. Kratz, 1963). According to the updated SANTE Directive (Document N^o SANTE/12682, 2019), the recoveries for the substances studied should range from 70 to 120% in all contaminated samples with relative standard deviations of $\leq 20\%$.

The SE method recovery rates of the substances studied for all concentrations ranged between ~60% and ~97%, except DMP which was not recovered at concentration 125μg Kg^{-1}, and DEP which was not recovered at all. The UAE method recovery rates for all concentrations of the 6 out of 7 substances studied ranged between ~60% and ~120%. DMP was not recovered at all at concentration 125μg Kg^{-1} and its recovery rates at 300 and 500μg Kg^{-1} ranged between 37% and 54%, which are quite low. Finally, the HS-SPME method recovery rates for all concentrations of 3 out of the 7 substances studied ranged between ~68% and ~103%. DEHP recovery rates for all concentrations ranged between 36.5% and 37.5%, which are low, and DOP and DMP were not recovered at all.

The accuracy of the methods was expressed as approximate trueness since Certified Reference Materials (CRMs) were not used in this study. The trueness of the methods was evaluated for a concentration of 125 μg Kg^{-1}. The concentrations were larger than 10 μg Kg^{-1} so the acceptable range was from -20% to + 10%.. A total of 20 tests were performed. The results are shown in **Table 1**; in bold are the concentration values within acceptable limits. According to the results, of the three methods, the HS-SMPE has the highest accuracy at 125 μg Kg^{-1} as 3 out of 7 contaminants are within limits, while in the UAE and SE methods only one out of 7 is within limits. The precision of the method was evaluated using the Horwitz Ratio (HorRat) equation (Horwitz & Albert, 2006). The experimental %RSD values, as well as the HorRat values for all 3 methods tested are given in **Table 2**. The results of both the RSD calculated values, as well as the HorRat values, lead to the conclusion that the HS-SPME is a more precise method for extracting the phthalates than the other two methods. All statistical data analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). The analysis of variance (ANOVA) was performed on 20 spiked samples in order to assess the differences between the average values for each of the studied compounds at concentration 125 μg Kg^{-1} and again the results showed that the HS-SPME was the most reliable method of the three. Having concluded that the HS-SPME method is the most reliable method of the 3 for the particular analysis, correction

factors were calculated to further optimize the accuracy of the results (**Table 3**). The correction factors were calculated by estimating the average value of 20 samples contaminated at concentration 125 μg Kg^{-1}. Finally, the concentrations of the contaminants in question that were determined in the 3 recycled paperboard materials are shown in **Table 4.**

Table 1. Approximate values of the 20 trials at concentration 125 μg Kg^{-1} for all three extraction methods.

Contaminant	UAE	SE	HS-SPME
DOP	75	75	-
DBP	88	92	86.13
DMP	-	-	-
DEP	**125**	-	**123.73**
DEHP	85	95	46.24
DEHA	81	**113**	**125.82**
BBP	150	75	**125.83**

Table 2. Experimental %RSD values and HorRat values for the UAE, SE, and HS-SPME methods at concentration 125μg Kg^{-1}.

Contaminant	UAE	SE	HS-SPME
DOP	1.45/0.07	1.55/0.07	-/-
DBP	2.22/0.10	1.84/0.08	1.09/0.05
DMP	-/-	-/-	-/-
DEP	1.96/0.09	-/-	1.15/0.05
DEHP	1.80/0.08	1.42/0.06	1.00/0.05
DEHA	1.28/0.06	1.62/0.07	0.93/0.04
BBP	1.93/0.09	1.14/0.05	0.98/0.04

Table 3. Correction factors calculated at concentration 125 μg Kg^{-1}.

Contaminant	DOP	DBP	DMP	DEP	DEHP	DEHA	BBP
Correction factor	-	1.45	-	1.01	2.70	0.99	0.99

Table 4. Concentrations found in the 3 paperboard samples used in this study and their corrected values

Contaminant	R1			R2			R3		
	Found (μg Kg^{-1})	SD	Corrected (μg Kg^{-1})	Found (μg Kg^{-1})	SD	Corrected (μg Kg^{-1})	Found (μg Kg^{-1})	SD	Corrected (μg Kg^{-1})
DOP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DBP	337	15	488	109	2	158	81	2	118
DMP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DEP	13	1	13	31	1	31	11	1	11
DEHP	139	2	375	189	8	510	193	2	521
DEHA	177	15	175	7	0	7	27	2	26
BBP	22	2	21	8	1	8	30	2	29

n.d.: not detected

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