

METHOD DEVELOPMENT FOR PHTHALATES DETERMINATION IN PRODUCTS FOR CHILDREN

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Abstract: Phthalates are plasticizers largely used in polyvinyl chloride (PVC) manufacture, nevertheless they are associated with risks to health. This study describes the method development for extraction of phthalates from vinylic plastics and quantification by GC-MS. This method will be applied for conformity assessment of products for children.

Key words: phthalates, safety, extraction, Soxhlet, GC-MS.

1. INTRODUCTION

Phthalates are plasticizers used to soften the polyvinyl chloride (PVC) for the manufacture of a wide variety of consumer products. However, they are associated with risks to health such as adverse reproductive effects, hepatic changes, and thyroid toxicity. Therefore, the presence of phthalates in toys, school or party supplies and childcare products has been a matter of concern. These chemical substances are being restricted in several countries, principally in products that can be taken into the mouth [1].

Brazil has restricted the presence of phthalates in vinylic parts of toys since 2007 [2]. This restriction is being now expanded for school supplies [3] and party supplies [4]. By May 2014, all school and party supplies sold in Brazil will have to comply with the acceptance criteria for phthalates. The following plasticizers are controlled by Brazilian regulation: di(2-ethylhexyl)phthalate (DEHP), dibutyl phthalate (DBP), and benzyl butyl phthalate (BBP). Moreover, products addressed for children under 3 must also comply with the criterion for diisononyl phthalate (DINP), diisodecylphthalate (DIDP), and di(n-octyl)phthalate (DNOP). In both cases, the sum of the phthalates must be less than 0.1 % in weight of plastic material [2,3,4]. Manufacturers must send their products to accredited laboratories for proving compliance with the specification. Figure 1 shows the chemical structure of these six phthalates.

Two general approaches are described in literature for extraction of phthalates from plastic matrixes: dissolve/precipitation process and solvent extraction. Extraction may be performed either at room temperature or with warm solvent in a Soxhlet apparatus. Quantification is commonly carried out by gas chromatography with flame ionization (GC-FID) or mass detection (GC-MS) [5,6].

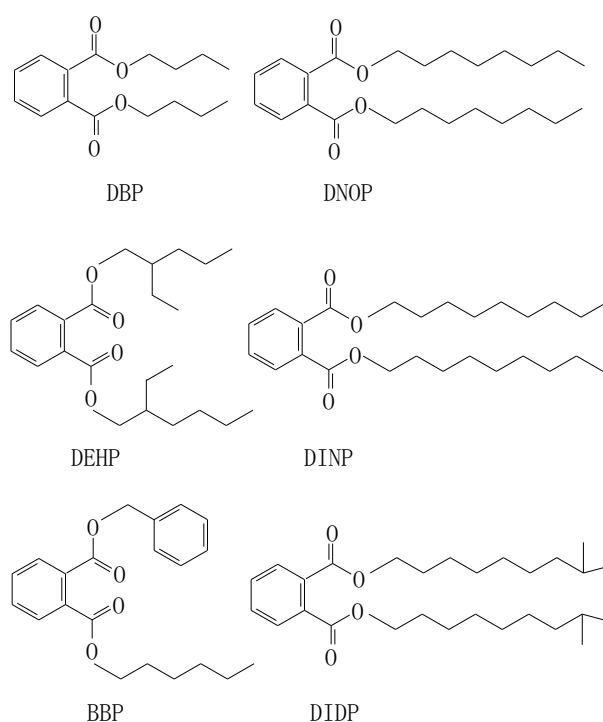


Fig. 1. Chemical structure of phthalates controlled in Brazil

2. PURPOSE

This study intends to develop a method for quantifying six phthalates in plastic matrices for further application in market surveillance for products addressed to children.

3. METHODS

DBP, BBP, DEHP, DNOP, DINP, DIDP and benzyl benzoate were purchased from Sigma-Aldrich. Chloroform (CHCl₃), dichloromethane (CH₂Cl₂), ethyl ether (Et₂O), methyl alcohol (MeOH) and tetrahydrofuran (THF) were obtained from Tedia.

Units of pen caps, toy cell phone and magic cube, positive for PVC in Beilstein test [7], were ground in a Willey-type mill from Marconi to produce a mixed sample. Each test was performed in triplicate with 2.0 g of sample. Soxhlet extractions (conditions are described in the next

section) were carried out in an automatic B-811 Büchi Extraction System, while extraction in CHCl_3 at room temperature and dissolution in THF were done under magnetic stirrer for 24 h. PVC dissolved in the last method (dissolution in THF) was precipitated by 2.5 volumes of MeOH. A second extraction was performed with a fresh portion of solvent for Soxhlet extraction methods. All the suspensions were filtered through cellulose filter, dried under nitrogen in Reacti-vap evaporator followed by drying at 105 °C for 30 min (dry extract), and dissolved in methanol. After proper dilution and addition of internal standard (IS), solutions were filtered through 0.45 μm nylon membranes.

The calibration curve was prepared in methanol in the concentration range of 0.5-56.0 $\mu\text{g/g}$ for DEHP, DBP, BBP and DNOP; and 5.0-560.0 $\mu\text{g/g}$ for DINP and DIDP (both are mixtures of isomers). Benzyl benzoate was used as IS at 30 $\mu\text{g/g}$.

The analysis was performed on an Agilent 6890N GC-MS with injector temperature 300 °C, injection volume 1 μL in split 1:5, Agilent DB1701 (30 m, 0.25 mm i.d., 0.25 μm film thickness) fused-silica capillary column under the following conditions: 160 °C held for 1 min, heated up to 280 °C at a rate of 10 °C/min and held for 9 min. Carrier gas was helium with a flow of 1.3 mL/min. Electron ionization was performed at 70 eV with the following MS conditions: source (230 °C), quadrupole (150 °C) and transfer line (280°C). The analytes were analyzed in selected ion mode (SIM) according to Table 1.

Table 1. Ions for SIM of phthalates and internal standard

Compound	Molecular mass	Corresponding ions (m/z) ¹
Benzyl benzoate (IS)	212	105 , 194, 212
Dibutyl phthalate (DBP)	278	149 , 167, 205, 223
Benzyl butyl phthalate (BBP)	312	91, 149, 206
di(2-ethylhexyl)phthalate (DEHP)	390	149, 167, 279
di(n-octyl)phthalate (DNOP)	390	149, 167, 279
diisononyl phthalate (DINP)	418	149, 167, 293
Diisodecylphthalate (DIDP)	446	149, 167, 307

¹Quantitative ions are in bold

4. RESULTS AND DISCUSSION

The total ion chromatogram (TIC) for the six phthalate esters is shown in Figure 2.

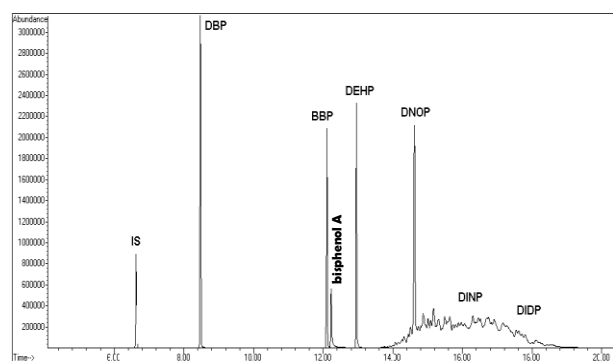


Fig. 2. TIC for the six phthalate esters

The DBP, BBP, DEHP and DNOP appeared as single peaks and DINP and DIDP appeared as isomeric mixture, providing large peaks. The major fragment measurement is at m/z 149 which is a result of the loss of alkyl ester groups. DNOP, DINP and DIDP partially co-eluted. Nevertheless, selectivity was achieved by using the ions for quantitative determination (m/z 279, m/z 293 and m/z 307, respectively). Figure 3 shows the SIM for DINP and DIDP in methanol solution.

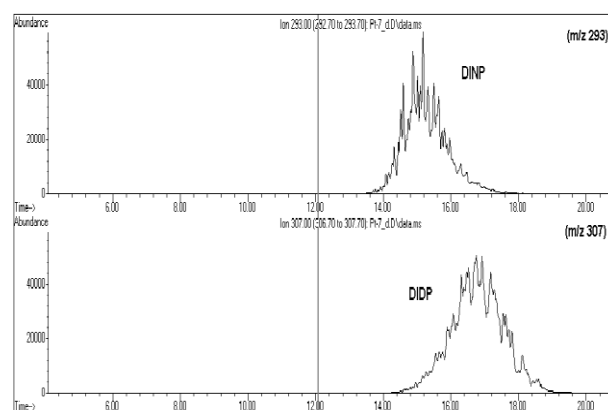


Fig. 3. SIM for the mixtures of isomers DINP and DIDP

Calibration curves were linear in the range of 0.5-56.0 $\mu\text{g/g}$ for DBP, BBP, DEHP and DNOP; and 5.0-560.0 $\mu\text{g/g}$ for DINP and DIDP. As indicated in table 2, all the phthalates achieved excellent linearity with coefficients of determination (R^2) larger than 0.995.

Table 2. Calibration curves

Phthalate	R^2	a	b	LoQ (%) ¹
DBP	0.9992	2.838	-0.097	0.0009
BBP	0.9986	0.265	-0.013	0.0009
DEHP	0.9984	0.153	-0.008	0.0009
DNOP	0.9976	0.210	-0.012	0.0009
DINP	0.9979	0.206	-0.108	0.0091
DIDP	0.9973	0.292	-0.199	0.0091

¹In terms of mass fraction, considering the PVC concentration in the sample around 55 mg/g

Extraction of phthalates from plastic matrix is a critical step in the analytical procedure for achieving suitable recovery. Provided there is no reference material for these substances in plastic matrix, studies were conducted in a comparative approach.

Three phthalates were present in the mixed sample: DBP, DEHP and DINP (Figure 4). BBP, DNOP and DIDP were found to be under the limit of quantitation (LoQ), which is 0.0009 % for BBP and DNOP, and 0.0091 % for DIDP (Table 2).

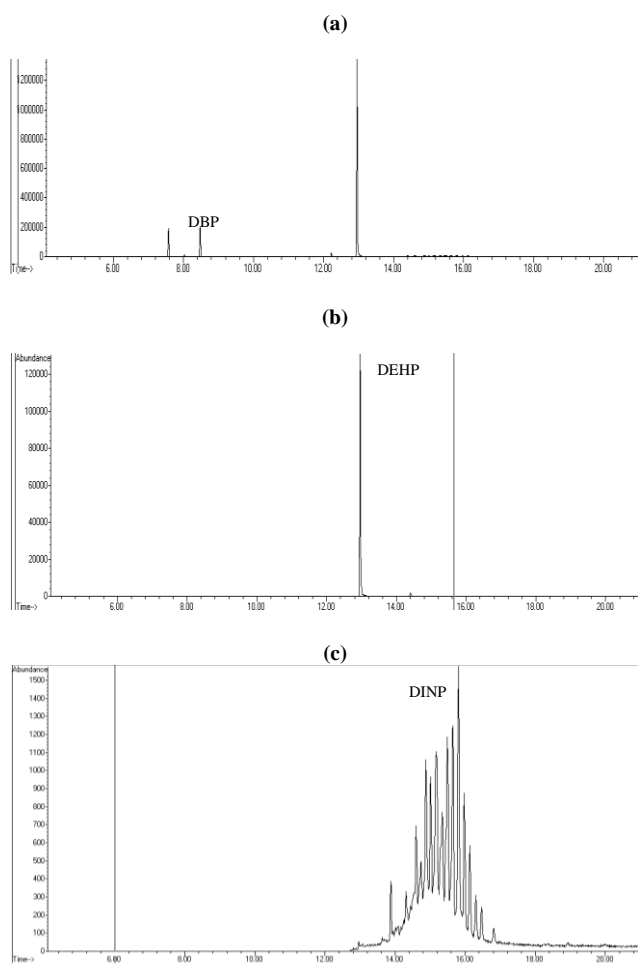


Fig. 4. SIM chromatograms for sample extracted with method 4: (a) DBP, m/z 149; (b) DEHP, m/z 279; and (c) DINP, m/z 293

Table 3 presents the conditions of the extraction methods. The results obtained in the main extraction (first) are shown in table 4, while table 5 shows the amount of phthalates extracted in the second cycle, when a fresh portion of solvent was added to the same sample.

Table 3. Methods used for phthalates extraction

Method ¹	Extraction ¹
1	Soxhlet, 6 h, 2:1 CHCl ₃ :MeOH
2	Soxhlet, 16 h, 2:1 CHCl ₃ :MeOH
3	Soxhlet, 6 h, Et ₂ O
4	Soxhlet, 6 h, MeOH
5	Soxhlet, 6 h, CH ₂ Cl ₂
6	Room temperature (RT) extraction, 24 h, CHCl ₃
7	Dissolution at RT, 24 h, THF, precipitation with MeOH

The use of single chlorinated solvent in methods 5 and 6 was observed to incidentally dissolve one type of plastic in the mixed sample, and this was confirmed once the dry extract of these tests (30.5 and 43.5 % w/w, respectively) was much larger than the sum of plasticizers detected. Besides the time consuming effort to clean out the PVC precipitated in sample cool down, it is clear in figure 2 that the high weight of dry extract in methods 5 and 6 is accompanied by a poor extraction of plasticizers. Once the second extraction for method 5 (Table 5) did not show relevant levels of extracted compounds, admittedly the substances were extracted in the first step, and thus they were present in the dry extract, but they were not dissolved totally in MeOH because they were incorporated into PVC sediment.

Table 4. Mass fraction (%) of phthalates extracted in the main extraction

Method	dry extract		DBP		DEHP		DINP		total		
	result	RSD (%)	result	RSD (%)	result	RSD (%)	result	RSD (%)	result	RSD (%)	relative (%) ¹
1	2.66	6.33	0.0067	3.96	0.0664	3.72	0.0441	1.52	0.1171	2.48	80
2	3.58	11.29	0.0077	2.36	0.0776	3.11	0.0474	1.93	0.1327	2.57	91
3	4.19	7.53	0.0067	6.70	0.0664	10.89	0.0435	2.62	0.1167	7.52	80
4	3.53	3.37	0.0086	0.56	0.0879	1.33	0.0500	1.88	0.1466	1.06	100
5	30.52	18.22	0.0028	21.78	0.0109	60.13	< LoQ	-	0.0138	52.20	9
6	43.51	37.92	0.0033	7.81	0.0181	7.59	0.0342	1.03	0.0557	3.55	38
7	2.37	18.47	0.0079	4.42	0.0888	4.78	0.0467	4.31	0.1434	4.56	98

¹Related to the total phthalates extracted in **method 4**, the method that present the highest extraction rate

Table 5. Mass fraction (%) of phthalates extracted in the second extraction

Method	dry extract		DBP		DEHP		DINP		total		
	result	RSD (%)	result	RSD (%)	result	RSD (%)	result	RSD (%)	result	RSD (%)	relative (%) ¹
2 ppt ²	0.20	39.63	< LoQ	-	0.0038	30.13	< LoQ	-	0.0038	30.13	2,89
1	0.34	21.59	< LoQ	-	0.0006	14.44	< LoQ	-	0.0006	14.44	0,55
2	0.21	53.13	< LoQ	-	0.0004	4.35	< LoQ	-	0.0004	4.35	0,28
3	0.98	52.06	< LoQ	-	0.0004	3.64	< LoQ	-	0.0004	3.64	0,32
4	0.14	4.12	< LoQ	-	0.0010	26.79	< LoQ	-	0.0010	26.79	0,67
5	2.73	24.42	< LoQ	-	0.0004	20.11	< LoQ	-	0.0004	20.11	2,98

¹Related to the total phthalates extracted in the **first extraction of the same method**

²Sediment that precipitated spontaneously when the first extract cooled down, and it was further prepared according to method 7 and analyzed

The room temperature extraction with CHCl_3 (method 6), which presented a poor performance in this study, was chosen by Wang and Storm (2005) for plasticizers and heat stabilizers extraction from PVC tubes [5]. In other words, extraction recovery is highly dependent on the properties of the matrix, thus a representative sample of polymers is important for developing a method suitable for different products.

The effect of co-precipitation of phthalates with PVC was also observed in a lower extension for method 2. In this test, MeOH diminished the PVC dissolution in the CHCl_3 -containing solvent. Even though, the PVC sediment that was formed when the extract cooled down presented significant amounts of plasticizers (2.9 % related to the first extraction).

The CHCl_3 :MeOH system was tested with 6 h (method 1) and 16 h (method 2) because the Brazilian technical regulation for party supplies specifies 16 h for this solvent, while for the alternatives Et_2O or CH_2Cl_2 (methods 3 and 5) the extraction time is 6 h [8]. According to the ANOVA test (Table 6, methods 1 and 2), the results obtained for 16 h of extraction were different from those acquired in 6 h, once F is higher than F crit and P-value is lower than alpha (0.05). Mass fractions of plasticizers for method 2 were 8 to 17 % higher than for method 1, depending on the compound. Therefore this system indeed requires a higher time of extraction.

Table 6. Two-factor ANOVA with replication for methods comparison¹ (95 % confidence level)

Methods	n	Between samples (methods)		
		P-value	F	F crit
1 and 2	9	2.77E-24	261.44	3.99
1 and 3	9	0.82	0.05	3.99
2, 4 and 7	6	4.48E-14	53.57	3.15
2 and 7	6	4.84E-08	44.96	4.08
4 and 7	6	0.12	2.50	4.08

¹F results for columns (DBP, DEHP, DINP and total phthalates) where higher than F crit for all the comparisons (data not shown)

The performance of Soxhlet extraction with Et_2O (method 3) was equivalent to method 1 (Table 6). Anyway, it did not offer advantages in terms of precision or potential for extraction when compared to other methods (Figure 5). Moreover, method 3 requires special care in the laboratory once Et_2O , a solvent prone to peroxide (explosive compounds) formation, must be heated and concentrated.

In the dissolve/precipitation process (method 7), one sample was considered outlier in Grubbs test and therefore it was excluded. In order to use the same degrees of freedom in ANOVA tests, when method 7 was present in the comparison one replicate of the other methods was randomly rejected resulting in 6 mass fraction data (two samples injected in triplicate).

The Soxhlet extraction with MeOH (method 4) offered the highest extraction rates for DBP and DINP, while the dissolve/precipitation process (method 7) was the best method for DEHP extraction. Anyway, according to ANOVA both methods were comparable in terms of DBP, DEHP, DINP and total phthalates extraction.

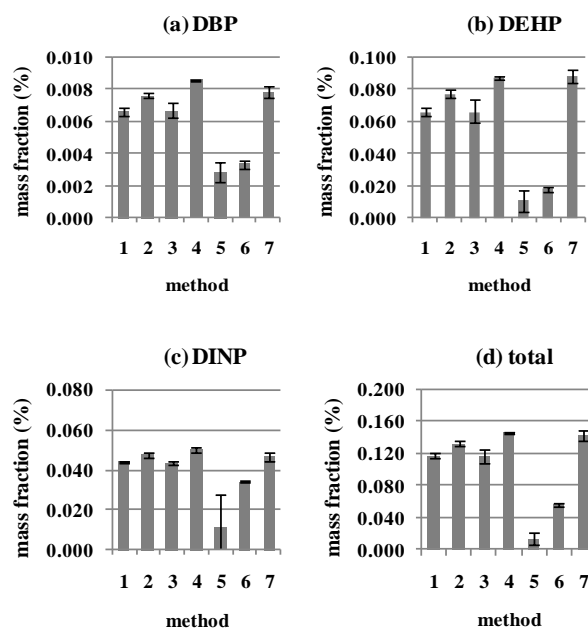


Fig. 5. Performance of extraction methods for phthalates; error bars show standard deviation; see Table 3 for method conditions

5. CONCLUSION

The use of MeOH for Soxhlet extraction of phthalates (method 4) was developed in this study and presented the best combination of extraction rate (recovery) and precision (RSD under 2.0 %). Furthermore, the dissolve/precipitation process (method 7) offered results equivalent to method 4, with the advantageous employ of a simpler and lower energy-consuming procedure. Provided the precipitation step is careful performed for avoiding outlier results, method 7 has a high potential for routine analysis. Methods developed in this study will be applied to assess conformity of products in a market surveillance approach.

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