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Development of an Extraction Method Using Mixture Design for the Evaluation of Migration of Non-target Compounds and Dibutyl Phthalate from Baby Bottles

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Abstract This work introduces a simple and rapid method for the extraction of baby bottle migrants from milk simulants employing a mixture of ethyl acetate:dichloromethane:hexane (27.5:22.5:50), using a simplex centroid design for optimization. Initially, the baby bottle materials were identified by FT-IR followed by migration test using 50% EtOH in water at 70 °C/2 h. Next, extraction and identification of migrants were performed by GC-MS. Furthermore, the additives in the baby bottle materials were quantified by HPLC-DAD. On the account of the toxicological potential of dibutyl phthalate, the optimized mixture was used for in-house validation by GC-MS of the proposed method. Dibutyl phthalate (DBP) migration was detected in three baby bottles with a concentration range of 175 to 235 μg kg^{-1}, which is lower than the specific migration limit determined by the Brazilian Health Regulatory Agency. However, exposure to DBP from baby bottles was estimated, and this was higher than the tolerable daily intake recommended by the European Food Safety Authority, indicating a potential public health concern.

Keywords Baby bottle · Polypropylene · Phthalate · Food contact materials · Migrants

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Introduction

Baby bottles are widely used to feed newborns and infants. In Brazil, the inclusion of milk in children's diet starts in the first month of life, reaching 18.2% in capitals and 50% by the end of the first 6 months (Brasil 2010; Simoneau et al. 2012). Due to the large use of baby bottles, the infants come in contact with different plastic materials, and chemical substances might migrate from the plastic into the foodstuff (Simoneau et al. 2012). The contamination of foodstuff with substances from the packaging material is a well-known issue. The presence of such contaminants can arise either from the packaging process itself (for example, via overprinting labels) or from migration processes. Furthermore, because infants are more sensitive to a variety of chemicals than adults, special attention must be paid to such aspects (Gärtner et al. 2009).

Most of these substances are additives, degradation products, or impurities from the raw material used for the manufacture of baby bottles. Recently, migration of chemical substances in baby bottles has been reported. Phthalate was identified among these substances using liquid–liquid extraction with ethyl acetate:n-hexane (1:1) or iso-octane before GC-MS analysis (Onghena et al. 2015; Onghena et al. 2014; Simoneau et al. 2012). These plasticizers have received special attention in the last years due to potential human toxicity. Experiments on animals report adverse health outcomes associated with phthalate exposure to the reproductive system, increase of asthma and allergies incidence, acute liver toxicity, and irritant effects (Sathyanarayana 2008).

Sampling techniques of the migrants analysis in simulants are, theoretically, quite simple. However, taking into account that organic compounds analysis is made by gas chromatography (GC), the direct injection of a simulant with high content of water, such as 50% ethanol, is not suitable. Moreover, migrants as phthalates are often in low concentrations (i.e.,

ng g^{-1}), requiring in some cases a concentration step before the analysis (Nerin et al. 2012).

Liquid–liquid extraction is, for this reason, the most used technique, besides being a fast and efficient technique. The drawback to this technique is the low selectivity and the large quantity of solvent required. In light of this, the selection of an extraction solvent is a crucial step when liquid–liquid extraction is applied. A good extraction solvent should have a strong solubility capability for the compounds of interest. On the other hand, compounds with different polarities cannot be extracted by a single solvent. This problem can be overcome using different liquid–liquid equilibrium systems of binary and ternary mixtures (Farajzadeh and Khoshmaram 2015).

Multivariate statistic tools, as mixture design, allow better visualization of interaction effects between the solvents assessed with a reduced number of experiments, in addition to reduction of time for analysis, costs, and the use of toxic solvents (Silveira et al. 2016). However, there is a paucity of published research focusing on the application of optimization, by experimental design, to migrants extraction methods from baby bottles. Most of the studies conducted univariate optimization for the extraction parameters.

Thus, this study was conducted in order to optimize and validate a fast and streamlined method for extraction and determination of migrants from baby bottles by GC-MS. The methods were applied to the migration testing of polypropylene (PP) and Tritan baby bottles. Moreover, the quantification of additives and the evaluation of phthalate migration as well as estimated intake from the use of baby bottles were performed.

Materials and Methods

Sampling

Twenty-three baby bottles, from four different models (A, B, C, and D), intended for children between 0 and 12 months were used in this work. From the four baby bottle models, 3 of them (D) were selected for the migrants extraction optimization, 12 were used for the migration tests, and 8 were used for the determination of additives profile. The baby bottles from model A were composed of Tritan™ (n = 5), and those from B, C, and D were composed of PP (n = 18), all of them with 70-mL capacity. The baby bottles were purchased from distributors located in São Paulo, Brazil.

Chemicals

Hexane, ethanol, acetone, iso-octane, dichloromethane, cyclohexane, and toluene, all HPLC grade, were purchased from Merck (DEU). Ultrapure water was obtained from a Direct-Q Millipore water purifier. Standards of dibutyl phthalate (Fluka; 99.6%), butylhydroxytoluene (Fluka; 99.9%), Irganox 1010 (Sigma-Aldrich; 98%), and Irgafos 168 (Sigma-Aldrich; 98%) were used for quantification.

Reducing Background

Taking into account that phthalate is a ubiquitous contaminant, any contact with plastic was avoided. All the glassware were thoroughly washed, rinsed twice with acetone and n-hexane, heated at 400 °C for at least 3 h, and finally stored in glass desiccators. Volumetric glasses were washed and rinsed with acetone, n-hexane, and iso-octane prior to use.

Confirmation of the Baby Bottle Materials

FT-IR (PerkinElmer) was employed to confirm the material of the baby bottles since the type of material was not always clearly indicated on the package. The analysis was conducted in the attenuated total reflectance mode. Spectra were measured with a resolution of 4 cm^{-1} in the range of 4000–650 cm^{-1}, each generated from 16 scans. The identification of polymer was made by comparing the spectrum obtained with the reference spectrum.

Development and Method Validation for Migrants Extraction

Three baby bottles of polypropylene (model D) were used for the development of extraction method. The procedure involved initial migration test simulating real use conditions of baby bottles. Therefore, the baby bottles were boiled for 5 min as recommended by the manufacturer, then filled with a milk simulant (i.e., 50% ethanol) at 70 °C, as specified in the Regulation 2011/10/EC and Brazilian Health Regulatory Agency—ANVISA (ANVISA 2010; European-Commission 2011).

The baby bottles filled with simulants were kept at 70 °C/2 h. Glass plates were used to cover the baby bottles to avoid simulant lost. The simulant was transferred into a pre-cleaned Erlenmeyer flask and allowed to cool to 25 °C before extraction. Second and third migration tests were performed according to the conditions for repeated use of food contact materials (ANVISA 2010; European-Commission 2011). Between the tests, the baby bottles were rinsed with ultrapure water and boiled again for 5 min.

Due to the incompatibility of the simulant with gas chromatography, a step was necessary to transfer the migrants from 50% ethanol to an organic nonpolar solvent. In order to identify the best solvent to apply in this step, a simplex centroid design (13 trials with 4 replicates in the central point) was performed (Table 1).

Mixtures of toluene:hexane, dichloromethane:hexane, and ethyl acetate:hexane, all in 1:1 (v/v) ratio, were evaluated. Incorporation of hexane in the extraction method was done

Table 1 Simplex centroid design

Trial	Toluene:hexane (X_1)		Dichloromethane:hexane (X_2)		Ethyl acetate:hexane (X_3)	
	Coded	Real (%)	Coded	Real (%)	Coded	Real (%)
1	1	100	0	0	0	0
2	0	0	1	100	0	0
3	0	0	0	0	1	100
4	0.5	50	0.5	50	0	0
5	0.5	50	0	0	0.5	50
6	0	0	0.5	50	0.5	50
7	0.66	66.7	0.16	16.7	0.16	16.7
8	0.16	16.7	0.66	66.7	0.16	16.7
9	0.16	16.7	0.16	16.7	0.66	66.7
10	0.33	33.3	0.33	33.3	0.33	33.3
11	0.33	33.3	0.33	33.3	0.33	33.3
12	0.33	33.3	0.33	33.3	0.33	33.3
13	0.33	33.3	0.33	33.3	0.33	33.3

in order to partition the organic and simulant solvents, since both ethyl acetate and dichloromethane are partially soluble in 50% ethanol.

Liquid–liquid extraction (LLE) was done in test tubes using 10 mL of the simulant from the first migration test and 2 mL of the mixtures assessed in Table 1. After, the extract was vigorously agitated for 20 s and cooled to 0 °C where the sample was maintained for 15 min. Afterwards, 1 μL of the organic phase was injected into gas chromatography coupled with a Triple-Axis Detector (Agilent 5975C inert) and electron impact ionization (GC-EI-MS).

The instrument was operated with a capillary column HP-5MS (30 m × 0.25 mm × 0.25 μm), injector block at 260 °C, helium as carrier gas at a constant flow rate of 1 mL min^{-1}, and a splitless injection. The oven program was set at 100 °C for 1 min, then 25 °C min^{-1} ramp to 310 °C for 3 min. Full scan analysis was used, monitoring ions between 50 and 550 Da. The MS spectra obtained from the migration tests was compared with spectra from NIST and Wiley libraries, observing concordance, at least 80% between the spectra.

All the compositions from the mixture designs, as well as "blank samples" prepared with a portion of the simulant, were analyzed by GC-MS in order to evaluate possible contaminations.

The optimal extraction condition, expressed as ion relative abundance, of migrants was assessed by the Derringer and Suich method (Derringer and Suich 1980). This method is based on the definition of a desirability function to each response, with values restricted to the interval [0,1], where "zero" means an unacceptable value and "one" the most desirable value. Once the desirability function has been set for all the answers, they should be combined into an overall desirability, normally given by the geometric average of "*n*" individual desirability.

The individual desirability was defined to maximize the ion abundance (by GC-MS). The mathematical models given by the desirability function were evaluated by variance analysis. The extraction condition optimization was performed using the Derringer and Suich algorithm with 95% reliability. The proposed mixture by the algorithm was experimentally validated and applied for the extraction of migrants from PP (n = 9) and Tritan™ (n = 3) baby bottles.

Quantification of Additives by HPLC-DAD

Quantification of additives from the baby bottle materials was carried out according to the adapted method from ASTM D6953-03 (ASTM 2009) in order to trace the source of migrants identified. Briefly, two baby bottles of each model were crushed to obtain the powder material. After homogenization, 5 g of the material was kept under reflux and agitation for 2 h with 50 mL of cyclohexane:dichloromethane (25:75, *v*/*v*).

Twenty-five milliliters of extract was concentrated to 5 mL. Ten microliters was injected into HPLC Agilent 1100 equipped with an automatic injector and a diode array detector operating at 200 nm. The separation was carried out in an RP-18 column (250 mm × 4 mm × 5 μm) using acetonitrile:water (95:5, *v*/*v*) as mobile phase in isocratic flux (1.5 mL min^{-1}).

In-house Validation, Quantification, and Dietary Intake Assessment of Dibutyl Phthalate

Taking into account all issues involving the phthalate toxicity, the dibutyl phthalate (DBP) quantification method was in-house validated according to recommendations of the harmonized guidelines and using the extract obtained in the migration test (Thompson et al. 2002). Validation parameters such as the limit of detection (LOD), limit of quantification (LOQ),

linearity, recovery, and precision were evaluated. Quantification was conducted in selective monitoring mode (SIM) by calibration curves in 50% ethanol, using BHT as the internal standard. The monitored ions for quantification were 205 and 220 to BHT and 149 and 223 to DBP.

Phthalate esters have been detected (ng L^{-1}) in organic solvents with high purity. These contaminants arise mainly in the manufacturing or storage of solvents in PVC tanks (Guo and Kannan 2012). Once phthalates are dispersed in the environment of analysis, LOD and LOQ are strongly dependent on the concentration of these compounds into the extraction solvent. Thus, the LOD was determined based on six injections (true replicates) of blank samples more than three times the standard deviation. The LOQ was set out as three times the LOD (Bratinova et al. 2009). Before setting out the LOD and LOQ, all solvents were analyzed to ascertain the presence of contamination.

Linearity was evaluated by calibration curve in 50% ethanol, with seven points (three replicates for each point, randomly injected). A DBP solution (2 mg kg^{-1}) was diluted in 50% ethanol in concentrations of 2.85, 150, 300, 450, 600, 750, and 900 μg kg^{-1}. The extraction was performed using 10 mL of the curve calibration solution and 2 mL of the optimized extraction solvent. Then, 1 μL of extraction solution was injected into the GC-MS (SIM mode) to obtain the related areas from each concentration. BHT (100 μg kg^{-1}) was added to the extraction solutions for food simulant as internal standard.

Lack of fit and linearity from the analytical curve was evaluated by analysis of variance (ANOVA). To reduce the instrumental background and avoid contamination, runs with the extraction solvent were carried out every six determinations. Moreover, the solvents used to wash the syringe were frequently replaced.

Recovery and precision (intra-day and inter-day) were assessed by blank spike with DBP (2.85, 400, and 900 μg kg^{-1}), with 3 replicates to recovery and 10 replicates during a day (intra-day) for three consecutive days (inter-day).

DBP daily intake was estimated according to the modified equation by Cirillo et al. (2015):

$$\text{Intake} = \frac{\sum(C \times V)}{\text{BW}}$$

where C is concentration, V is volume of the milk simulant used in the migration tests (630 mL), and BW is body weight.

Estimative was done taking into account babies that are 1 to 6 months old. DBP levels were determined from three baby bottles and the weight of babies at the 50th and 97th percentile according to the growth curve by the World Health Organization (WHO 2006). We considered a scenario that three new baby bottles were used to feed the babies in a single day.

Results and Discussion

Confirmation of the Baby Bottle Materials

Tritan™ is composed of three monomers: dimethyl terephthalate (DMT), 1,4-cyclohexanedimethanol (CHDM), and 2,2,4,4-tetramethyl-1,3-cyclobutanediol (TMCD) (Osimitz et al. 2012). The spectrum of the baby bottles (model A) (Fig. 1) shows bands at 726, 771, and 872 cm^{-1}, indicating the presence of benzene groups present in DMT. The band at 872 cm^{-1} indicates a disubstituted group at positions 1 and 4. The bands at 1017 and 1096 cm^{-1} are attributed to the C–O stretch from alcohol groups present in both TMCD and CHMD. The band at 1700 cm^{-1} corresponds to carbonyl groups, and the bands at 2924 and 2854 cm^{-1} indicate alkene stretch. In light of this, the characteristics obtained in FT-IR analysis revealed that the baby bottles were made of Tritan™.

Baby bottle spectra from models B, C, and D clearly revealed characteristic bands from polypropylene: bands at 809, 900, and 973 cm^{-1} are related to the C–C anti-symmetric and symmetrical stretch; deformation of the methyl group is characterized by bands at 841, 899, 956, 973, 997, 1375, 1455, 2838, 2867, 2917, and 2950 cm^{-1}. The band in the region of 1700 cm^{-1} may be formed from thermal degradation of PP or any additive used during the polymer manufacturing process (Tadokoro et al. 1965).

Development and Method Validation for Migrants Extraction

Studies approaching mixture design for migrants extraction from food contact materials, like baby bottles, are still scarce. The few published studies in this subject perform the extraction procedure by using standard or target substances, which makes the method poorly selective when it comes to non-target substances that might be extracted during the migration test.

In the proposed method by our work, nine substances were extracted and tentatively identified from baby bottles D (Table 2), among them are alkanes, phthalates, and alkyl esters. However, only dibutyl phthalate is registered as a restricted use substance by ANVISA (2008).

Though polypropylene does not have phthalates in its composition, phthalates might be derived from polymerization or/and pigmentation steps. Other substances may come from the resin degradation process or even from impurities present in the raw material used in manufacturing baby bottles. In most cases, these substances do not cause harm to human health, because they occur at low concentrations. However, there is no guidance about what should be done when an unknown compound is detected in food contact materials, such as in the case of baby bottles analyzed in this work (Nerin et al. 2013).

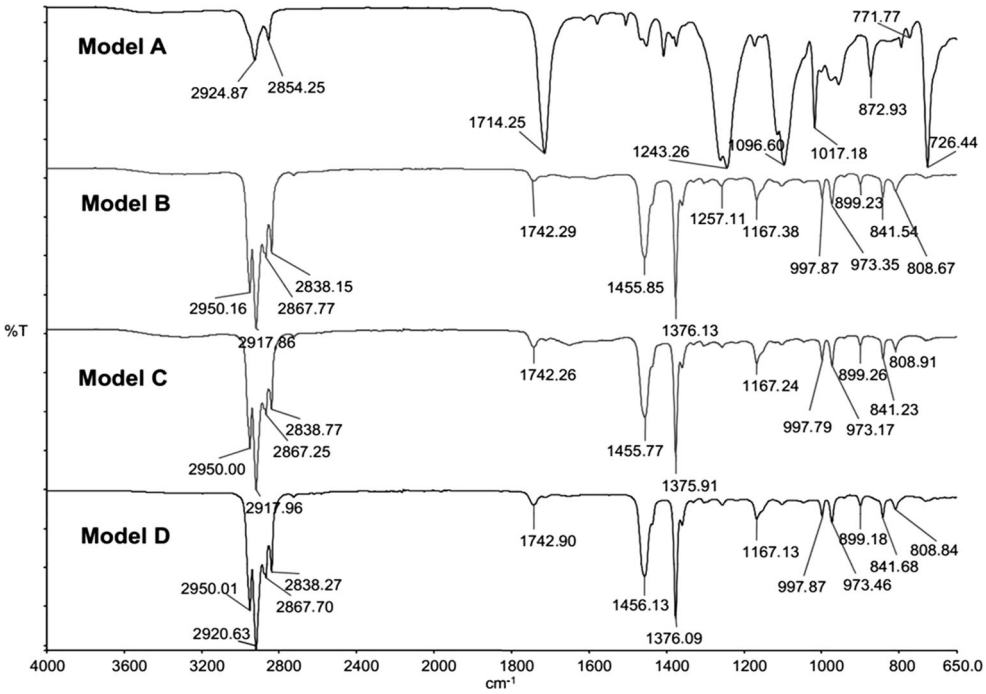

Fig. 1 FT-IR spectrum from baby bottles

Results obtained for the mixture design were compared by analysis of variance (ANOVA) and were considered to be significantly different when $p < 0.05$. A model was adjusted to all compounds, except for dodecyl acrylate, hexadecanoic acid, methyl ester, and methyl cis-9,10-epoxyoctadecanoate (Table 2). In the face of several responses and different predicted extraction conditions, a tool for predictive modeling (Derringer and Suich) was used for simultaneous response optimization and to find the best capable condition to extract all the identified compounds. Only one condition was able to extract the nine compounds with desirability of 0.80.

The optimized condition was composed of a mixture of ethyl acetate (27.5%), dichloromethane (22.5%), and hexane (50%). Three confirmatory tests were performed to experimentally evaluate the best condition given by the model. Good concordance between the predicted values (for the extraction conditions and response, as peak areas) and the experimentally obtained ones can be observed in Table 3.

Except for dodecyl acrylate, all compounds showed peak areas in an expected range based on the Derringer and Suich desirability function (Table 3). Although acrylate has already been reported in PP baby bottles (Onghena et al. 2014), this compound is not used in PP production. Moreover, regression models that are not significant and/or show lack of fit, as for dodecyl acrylate, cannot be accurately predicted by the Derringer and Suich function.

The optimized mixture was used for extraction of migrants in order to tentatively identify them by GC-MS in baby bottles of models A, B, C, and D. Some of these compounds (Table 4) are not prescribed for use in plastic food contact materials (ANVISA 2008; ANVISA 2012). However, these substances may not be listed because they are PP degradation products, such as aliphatic hydrocarbons octadecane and pentadecane, and also branched chain compounds, such as 3-ethyl-3-methyl-decane, which can be formed either by PP processing or by material aging process (Espert et al. 2005).

2,4-bis(1,1-dimethylethyl)-phenol was regarded before as a degradation product from Irgafos 168 (Cherif Lahimer et al. 2013). 2,4-bis(1,1-dimethylethyl)-phenol is an antioxidant that can be used in PP, and it is not listed as a substance restricted for use (ANVISA 2008; European-Commission 2011).

Phthalic acid esters, such as diisobutyl phthalate (DIBP) and dibutyl phthalate (DBP), are added to plastics to make it soft and flexible (Jurewicz and Hanke 2011). The migration of these compounds from baby bottles has been related to concentrations below the specific migration limit (Onghena et al. 2016; Simoneau et al. 2012). However, some phthalates, such as DIBP, cannot be used as an additive in food contact materials. Conversely, DBP can be used and has a specific migration limit of 0.3 mg/kg (ANVISA 2008; European-Commission 2011).

Hexadecanoic acid, methyl ester, pentadecanoic acid, methyl ester, and 9-octadecenoic acid (Z)-methyl ester may be derived from ethyl hexadecanoate that is used as a lubricant, even methyl stearate, to increase the mechanical strength and modify the rheological properties of polymer melt. They are applied in bulk or in the surface of the plastic to reduce friction and increase its flexibility (Cherif Lahimer et al. 2013). Moreover, the

Table 2 Model significant coefficients and ANOVA ($p < 0.05$)

Substances	Model	Regression	Model Fit	Significant coefficients ± standard error						
		$P < 0.05$	$P > 0.05$	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	$X_1X_2X_3$
Octadecane	Cubic	0.002	0.8901	464,418	843,311	1,270,895	–	–	−1,348,858	5,467,953
Dodecyl acrylate[a]	Quadratic	0.1323	0.415	804,629	907,299	801,248	−245,336	486,069	525,624	–
Diisobutyl phthalate	Quadratic	<0.0001	0.603	3,496,282	4,264,369	6,317,785	–	–	–	–
Hexadecanoic acid, 15-ethyl-, methyl ester	Quadratic	0.004	0.872	553,456	2,483,401	2,150,436	–	–	–	–
Dibutyl phthalate	Quadratic	0.003	0.520	20,478,017	23,929,475	23,146,808	–	14,358,471	17,608,555	–
Hexadecanoic acid, methyl ester[b]	Linear	<0.0001	0.007	1,045,206	3,323,545	2,261,808	–	–	–	–
Methyl stearate	Linear	<0.0001	0.727	1,230,410	1,338,978	1,156,737	−1,975,824	773,671	906,688	–
Heptadecanóico acid, ethyl ester	Linear	0.001	0.188	3,336,902	9,216,856	7,141,169	–	–	–	–
Methyl cis-9,10-epoxyoctadecanoate[a]	Linear	0.076	0.912	2,599,158	2,834,982	3,885,569	–	–	–	–

[a] Regression model not significant ($p < 0.05$)

[b] Regression model with lack of fit ($p < 0.05$)

carboxylic acids and derivatives can be produced from polyolefin oxidative degradation (Contat-Rodrigo et al. 2001; Espert et al. 2005).

Dimethyl terephthalate is applied as monomers for production of Tritan™, and its use is not restricted according to Regulation 10/2011/EC (European Commission) and 56/2012 (ANVISA). Benzoic acid, 4-ethoxy-,ethyl ester can be applied as additive or processing aid in polyolefins with specific migration quantity of 3.6 mg/kg (ANVISA 2012; European-Commission 2011).

Diphenylethanedione, methyl cis-3-ethyl oxirane octanoate, 1,1′-(6-methoxy-2,5-benzofurandiyl) bis-ethanone, and hexamethylene diacrylate have been identified in baby bottles but might be degradation products. For this reason, these are not described in the positive list of substances that can be added to food contact materials (ANVISA 2008; ANVISA 2012; European-Commission 2011).

Quantification of Additives

The quantification and identification were performed and compared by using both retention index and absorption specters from compounds and standard solutions.

All the PP baby bottles have in their composition Irganox 1010 and Irgafos 168, two antioxidants usually employed in polyolefins with no restrictions on the use (ANVISA 2008; European-Commission 2011). The concentration of these compounds varied between 398 and 438 mg kg^{-1} for Irganox 1010 and between 636 and 777 mg kg^{-1} for Irgafos 168.

Another substance was detected, but it was not possible to be identified by HPLC-DAD. The compound is probably phenol, 2,4-bis(1,1-dimethylethyl), which is formed from Irgafos 168 and was also detected in the non-target analysis (Dopico-García et al. 2007).

Baby bottles from model A could not be analyzed by this method because the solution formed after the contact of extraction solvent and the Tritan™ was highly viscous. This behavior may be associated with the change in the polymer structure once cyclohexane has great chemical similarity with cyclohexanedimethanol, a monomer present in Tritan™ composition.

Polyolefin polymer may undergo degradation through oxidation mechanism by air and UV light (Jeon et al. 2007). For this reason, the addition of these compounds to polypropylene is common.

The determination of antioxidant concentrations in polyolefins provides information about the potential migration. In general, the studies are focused on few compounds that are more commonly used, such as the phenolics Irganox 1010 or Irganox 1076 and the phosphites Irgafos 168 (Dopico-García et al. 2007; Lin et al. 2011).

Table 3 Predicted values after optimization by the Derringer and Suich desirability function

	Predicted value		Mean of observed values
	Limit inf.	Limit sup.	
Toluene:hexane (TOL)	0	1	–
Dichloromethane:hexane (DM)	0	1	–
Ethyl acetate:hexane (ACET)	0	1	–
Octadecane	745,201	1,080,255	929,770 ± 48,352
Dodecyl acrylate	777,351	1,070,081	722,876 ± 159,915
Diisobutyl phthalate	4,357,410	5,047,024	4,394,039 ± 371,963
Hexadecanoic acid, 15-ethyl-, methyl ester	813,728	2,655,113	814,421 ± 61,957
Dibutyl phthalate	23,626,680	28,228,006	24,192,125 ± 1,388,709
Hexadecanoic acid, methyl ester	1,664,550	2,763,934	2,137,591 ± 241,794
Methyl stearate	1,086,550	1,337,772	992,639 ± 114,150
Heptadecanóico acid, ethyl ester	4,613,516	8,541,797	5,851,508 ± 795,172
Methyl cis-9,10-epoxyoctadecanoate	2,050,472	4,171,244	2,132,803 ± 545,222

In-house Validation Method for DBP Quantification and Risk Assessment

Limits of detection (LOD) and quantification (LOQ) for the analysis of phthalates are strongly dependent on blank concentration. These limits are usually determined as the mean concentration value for the blank more than 2 or 3 times the standard deviation (Fierens et al. 2012). In this work, the LOD and LOQ were of 0.95 and 2.88 μg kg^{-1}, respectively.

Blank values are the results from an analytical read, or the values come from the use of reagents, solvents, chemicals, glassware, and materials that come in contact with the samples in the analytical process and any residual contamination in the measuring device or during analysis process that may contribute to the final value (Wenzl 2009). In spite of the fact that contact with plastic materials has been avoided and several methods have been reported to reduce the contamination with phthalates, like distillation and addition of adsorbents, blank problems are common in the phthalate analysis, and the air is the main source of the contamination (Fankhauser-Noti and Grob 2007; Wenzl 2009).

Based on the analysis of variance (ANOVA), we observed significant linear regression in the range of 2.88 and 900 μg kg^{-1} and good fit for the regression model ($p > 0.05$),

Table 4 Non-target compounds identified by GC-MS (quality index >80%)

Substances	CAS	% quality index	Model A	Model B	Model C	Model D
Octadecane	629-92-5	84				×
Phenol, 2,4-bis(1,1-dimethylethyl)-	9676-4	93				×
Ethanedione, diphenyl-	13481-6	90				×
Diisobutyl phthalate	8469-5	94	×	×	×	×
Hexadecanoic acid, methyl ester	11239-0	91		×		×
Dibutyl phthalate	8474-2	95				×
Pentadecanoic acid, ethyl ester	41,114-00-5	83		×		×
Methyl stearate	11261-8	87				×
Methyl cis-9,10-epoxyoctadecanoate	2500-59-6	80				×
Octadecenoic acid, ethyl ester	106-33-2	84	×	×	×	×
Pentadecane	629-62-9	83		×		
Decane, 3-ethyl-3-methyl-	17,312-66-2	82		×		
Ethanone, 1,1′-(6-methoxy-2,5-benzofurandiyl)bis-	23,840-15-5	80		×		
9-octadecenoic acid (z)-, methyl ester	112-62-9	87		×		
Dimethyl terephthalate	120-61-6	90	×			
Benzoic acid, 4-ethoxy-, ethyl ester	23,676-09-7	84	×			
Hexamethylene diacrylate	13,048-33-4	86	×			
Ethyl hexadecanoate	628-97-7	83	×			

Table 5 Migration of DBP (μg kg^{-1}) from baby bottles

Model	A	B	C	D
1° use	<LOQ	< LOQ	< LOQ	235.30 ± 25.31
2° use	< LOQ	< LOQ	< LOQ	178.10 ± 6.24
3° use	< LOQ	< LOQ	< LOQ	175.89 ± 8.16

$N = 3$

LOQ limit of quantification

which allows us to use the curve for quantification. Intra-day and inter-day precisions, expressed as relative standard deviation (%RSD), ranged from 3.7 to 8.9% and from 3.70 to 15.96, respectively. Recovery was evaluated through spike of blank solutions at three concentration levels of the curve, i.e., LOQ, 450 and 900 μg kg^{-1}, and ranged from 90 to 114%.

The method was used to evaluate the phthalate migration in 12 baby bottles. In only one model of the baby bottles, DBP migration was detected (Table 5). As baby bottles are repeatedly used utensils, the test was done three times on the same sample using a new simulant in each time (ANVISA 2010).

DBP (CAS 084-74-2) specific migration limit is 0.3 mg kg^{-1}; therefore, the DBP concentration that migrated from baby bottles was in compliance with ANVISA and the Regulation 10/2011/EC from European Commission (ANVISA 2010; European-Commission 2011). Onghena et al. (2014) and Simoneau et al. (2012) reported lower concentrations for DBP migration than those found in this study. However, phthalates can only be added in materials or reusable objects that are not in contact with fatty foods. In this sense, phthalates could not be used in baby bottles since the main use of these utensils is to containing and carrying milk which is rich in fat (ANVISA 2008; European-Commission 2011).

The high concentration of phthalate in baby bottles from model D can be due to the polymerization process or pigment use in the polymer. Castle et al. (1989) reported DBP migration in bakery products covered with pigmented PP and found a high correlation between the plasticizers detected in the printing ink of the films and in the contaminated food.

Regarding polymerization process, the Ziegler-Natta catalyst uses DBP as an internal donor of protons, which can increase the production of isotactic polypropylene. Isotactic polypropylene is the most commercialized PP form, due to high crystallinity and chemical and overheating resistance. Thus, this polymerization process to produce isotactic polypropylene might be also used in obtaining baby bottles (Makwana et al. 2009).

DBP intake was estimated for babies 1 and 6 months old, taking into account that milk is the only food introduced for this group. The European Food Safety Authority (EFSA) established a tolerable daily intake (TDI) of 10 μg/kg bw for DBP (EFSA 2005). The highest intakes of DBP were estimated among infants with growth at the 50th percentile who have lower body weight than those at the 97th percentile (Fig. 2). Daily intake of DBP varied between 12.67 and 27.5 μg/kg bw, reaching up to 175% the TDI for babies in the 50th percentile. Similar values of DBP daily intake have been reported by Cirillo et al. (2015) from an infant formula. These DBP concentrations only would be safe for 2.1-year-old babies in the 50th percentile or for 1.2-year-olds in the 97th, both at 12.4 kg. Furthermore, babies do not have the same biotransformation and elimination mechanisms with adults, which might increase their exposure to DBP.

Many studies have reported the toxic effects of phthalate exposure (Factor-Litvak et al. 2014; Howdeshell et al. 2015; Sathyanarayana 2008). Recently, studies have shown that DBP may cause some neurobehavioral adverse effects in mice, reducing the total distance movement, impairing memory function, and inducing anxiety (Farzanehfar et al. 2016). For this reason, the efforts should be directed to avoid or

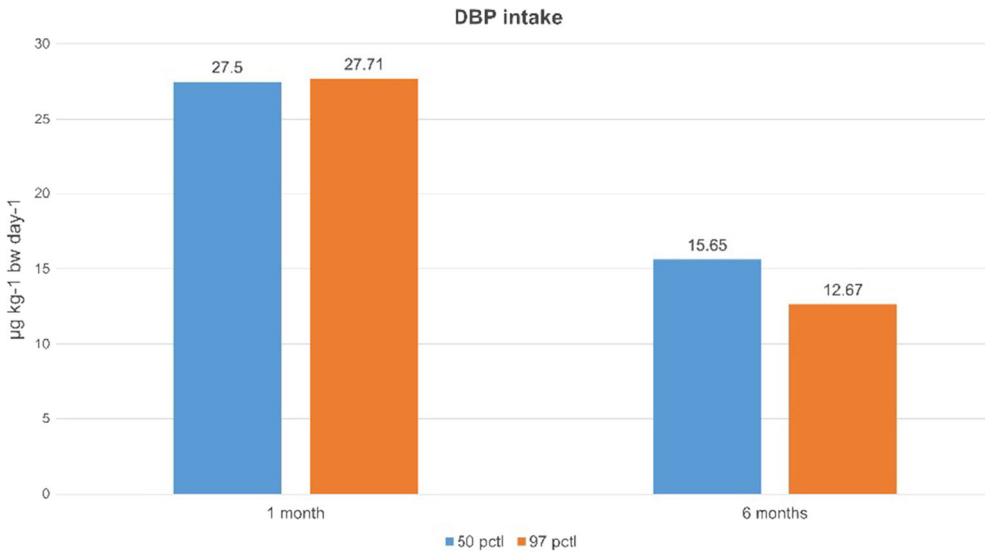

Fig. 2 Estimated DBP intake

reduce the use of phthalates either in baby bottles or in any utensil for babies.

Conclusion

Our data have shown that a mixture of ethyl acetate:dichloromethane:hexane (27.5:22.5:50) is extremely effective for the extraction of non-target compounds and dibutyl phthalates from milk simulants (50% ethanol). Irganox 1010 and Irgafos 168 have been detected in the material of all PP baby bottles. Regarding phthalate migration, only DBP was quantified in baby bottle D, with a migration lower than that established by ANVISA. However, the dietary intake assessment has shown that the DBP tolerable daily intake might reach 175% of that recommended by EFSA. In view of this, the incidence of DBP should be monitored, since the main exposure route of children is through intake of contaminated food or by using materials containing it, such as baby bottles.

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Compliance with Ethical Standards

Conflict of Interest Wellington da Silva Oliveira declares no conflict of interest. Thais Cristina Lima de Souza declares no conflict of interest. Marisa Padula declares no conflict of interest. Helena Teixeira Godoy declares no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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