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DETERMINATION OF PHTHALATE MIGRATION FROM CHILDREN'S TOYS INTO ARTIFICIAL SALIVA USING A QUECHERS-BASED METHOD AND GC/MS ANALYSIS

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INTRODUCTION

Phthalates are chemical plasticizers that are widely used to alter the properties of polymers, such as increased softness and more flexibility. Some phthalates are endocrine disruptors, possible carcinogens and may cause reproductive defects. Plastics containing phthalates are commonly used in the manufacture of children's toys and since phthalates are not chemically bound to plastics they may leach into saliva through touching, licking and chewing. To ensure child safety, it is important to quantitatively determine phthalate migration from plastic toys into saliva.

The QuEChERS sample preparation approach was introduced in 2003 by Anastassiades and Lehotay et al. for the determination of pesticide residues in fruits and vegetables [1]. QuEChERS involves the extraction and partitioning of residues from samples with high water content (Dry matrices need to be hydrated by water before extraction) into acetonitrile (MeCN) with the addition of salts to induce phase separation. This is followed by dispersive solid phase extraction (dSPE) to remove matrix co-extractives. Since its inception QuEChERS has been widely used for the analysis of pesticide residues and has been adapted for a wide range of chemical residues and matrices.

The aim of this study was to develop a simple, fast and reliable QuEChERS-based method to determine the level of phthalates that leach out of toys and into artificial saliva. Ethyl acetate (EtOAc) was used as extraction solvent instead of MeCN. No dSPE cleanup was employed as the artificial saliva and toy samples were found to contain less matrix co-extractives compared to food samples. The identification and quantitation of phthalate residues was achieved by GC/MS.

EXPERIMENTAL

MATERIALS:

50 mL polypropylene centrifuge tubes (ECPAHFR50CT) and QuEChERS extraction salts (4 g magnesium sulfate and 2 g sodium chloride) packed in a Mylar pouch (ECOUUS2-MP) were supplied by UCT (Bristol, PA). Pesticide grade ethyl acetate (EtOAc) was purchased from Spectrum (New Brunswick, NJ). Neat xanthan gum, potassium chloride, sodium chloride, magnesium chloride, calcium chloride and di-potassium hydrogen orthophosphate were purchased from Sigma-Aldrich (St. Louis, MO). A 1000 ppm semi-volatile mixture in methylene chloride containing 6 phthalates was purchased from Restek (Bellefonte, PA). A 5000 ppm triphenyl phosphate (TPP) standard in methyl tert-butyl ether was purchased from Cerilliant (Round Rock, TX).

PREPARATION OF STANDARDS AND ARTIFICIAL SALIVA:

A 5 ppm phthalate spiking solution was prepared by mixing 25 µL of the 1000 ppm semi-volatile mixture with 4.975 mL of EtOAc. A 50 ppm TPP internal standard (IS) was made by mixing 50 µL of the 5000 ppm TPP standard with 4.95 mL of EtOAc.

Artificial saliva (AS) was prepared by weighing 0.18 g of xanthan gum, 1.2 g of potassium chloride, 0.85 g of sodium chloride, 0.05 g of magnesium chloride, 0.13 g of calcium chloride and 0.13 g of di-potassium hydrogen orthophosphate into a 1-L volumetric flask, diluting to volume with Milli-Q water, and stirring for 4 hours [2].

PROCEDURE:

1. Cut toy samples into small pieces. Weigh 1.77 g into a 50-mL polypropylene tube and add 10 mL of AS and 2 stir bars. For blank and fortified samples, add 10 mL of AS and 2 stir bars to a 50-mL tube, spike with appropriate amounts of the 5 ppm phthalate spiking solution.
2. Shake tubes for 1 hour using a horizontal shaker.
3. For toy samples only: transfer AS into new 50-mL polypropylene tubes.
4. Add 10 mL of EtOAc to all tubes and shake for 1 min by hand.
5. Add salts in Mylar pouch, shake vigorously for 1 min and centrifuge at 5000 rpm for 5 min.
6. Transfer 1 mL of the supernatant into a 2-mL autosampler vial, add 10 µL of the 50 ppm TPP (IS) and vortex for 30 sec.
7. The samples are ready for GC/MS analysis.

PREPARATION OF MATRIX-MATCHED CALIBRATION STANDARDS:

Matrix-matched calibration standards were prepared by spiking 0, 25, 50, 100, 200, and 1000 ng/mL of phthalates to extracts obtained from blank artificial saliva sample that underwent the whole extraction procedure.

INSTRUMENTAL:

GC/MS: Agilent 6890N GC equipped with a 7683 auto sampler and coupled to a 5975C MSD
Injector: 1 µL splitless injection at 250 °C, 30 mL/min split vent at 1 min
Liner: 4 mm splitless gooseneck, 4mm ID*6.5mm OD*78.5mm (GCLGN4MM) packed with deactivated glass wool
GC column: Restek Rxi®-5sil MS, 30m*0.25mm*0.25µm integrated with 10m guard column
Oven temperature program: Hold initial temperature of 70 °C for 1 min; ramp at 20 °C/min to 315 °C and hold for 4.75 min. Acquire data from 6 to 14 min.
Carrier gas: Ultra high purity helium at a constant flow of 1.2 mL/min.
MSD: Transfer line @ 280 °C; MS Source (ESI+) @ 250 °C; MS quad @ 150 °C
Scan range: 55-350 amu
Dwell time: 25 ms

GC/MS SIM parameters

Compound	Abbreviation	Rt (min)	Group #	Start (min)	Quantify ion	Qualifier ion 1	Qualifier ion 2
Dimethyl phthalate	DMP	6.989	1	6.0	163	194	133
Diethyl phthalate	DEP	7.858	2	7.5	149	177	105
Dibutyl phthalate	DBP	9.865	3	9.0	149	223	150
Benzyl butyl phthalate	BBP	11.716	4	11.0	149	91	206
Triphenyl phosphate	TPP	11.964			326	325	
Bis(2-ethylhexyl) phthalate	DEHP	12.432	5	12.3	149	167	279
Di-n-octyl phthalate	DOP	13.138			149	279	150

RESULTS

LINEARITY AND LIMIT OF QUANTITATION:

The responses were linear over the calibration range with correlation coefficient (R2) greater than 0.9973. The limit of quantitation (LOQ) of the method was found to be 25 ng/mL for all six analytes.

Compound	Linearity range (ng/mL)	R²
Dimethyl phthalate	0-1000	0.9992
Diethyl Phthalate	0-1000	0.9999
Dibutyl phthalate	0-1000	0.9995
Benzyl butyl phthalate	0-1000	0.9973
Bis(2-ethylhexyl) phthalate	0-1000	0.9999
Di-n-octyl phthalate	0-1000	0.9998

ACCURACY AND PRECISION:

AS samples fortified with 200 and 500 ng/mL phthalates were extracted with the QuEChERS-based procedure. Recoveries ranging from 85.5 to 97.0% and relative standard deviations (RSDs) less than 3.8% were achieved, indicating that this method is accurate and precise to determine phthalate migration from toys into artificial saliva.

Accuracy and precision results of artificial saliva fortified at 2 concentrations

Compound	Fortified at 200 ng/mL		Fortified at 500 ng/mL	
	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)
Dimethyl phthalate	90.2	2.6	94.6	2.1
Diethyl Phthalate	91.1	1.4	95.6	2.0
Dibutyl phthalate	90.6	3.8	97.0	2.2
Benzyl butyl phthalate	85.5	1.2	92.1	2.5
Bis(2-ethylhexyl) phthalate	93.2	2.2	92.7	2.6
Di-n-octyl phthalate	88.8	3.1	92.8	1.3

APPLICATION TO REAL SAMPLE:

A real toy sample (weighed 5.31 g) was cut into small pieces (< 1cm*1cm) and extracted in triplicate (1.77 g each) using the newly developed method. One phthalate (diethyl phthalate) was detected at 50.5 ng/mL (5.9% RSD, n=3) in the AS (Fig. 3), which corresponds to 285 ng/g of diethyl phthalate leached from the toy sample into the AS.

CALCULATION:

50.5 ng/mL * 10 mL / 1.77 g = 285 ng/g
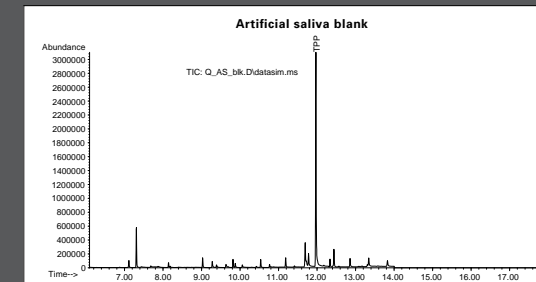
CHROMATOGRAMS:

Figure 1. Chromatogram of a blank artificial saliva sample
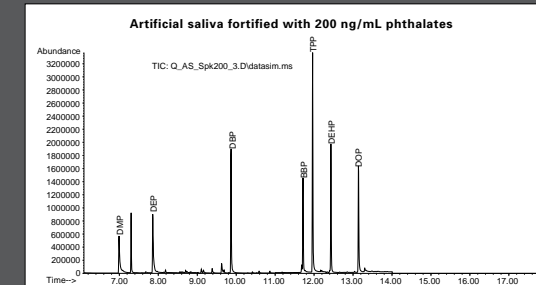

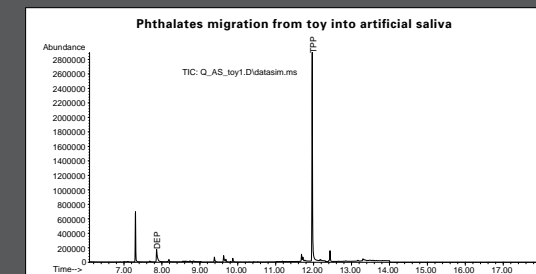Figure 2. Chromatogram of artificial saliva fortified with 200 ng/mL phthalates

Figure 3. Chromatogram of a real toy sample found to be positive for diethyl phthalate (DEP).

CONCLUSION

- This poster presents a procedure that artificially reproduces children licking and chewing in order to determine the level of phthalate residues that leach from plastic toys into artificial saliva.
- Phthalate residues were determined using a simple analytical method consisting of a fast extraction with EtOAc followed by GC/MS analysis.
- Satisfactory recoveries ranging from 85.5 to 97.0% and with RSDs ≤ 3.8 % were obtained from samples fortified at two concentrations (200 and 500 ng/mL; n=4).
- The analytical method was linear over a 0 to 1000 ng/mL concentration range and LOQs were found to be 25 ng/mL for all six phthalates included in this study.
- The developed method was subsequently applied to a real sample. Diethyl phthalate was found to leach out of the toy sample and into the artificial saliva at a concentration of 285 ng/g.
- The results of this work indicate the importance of monitoring for phthalate migration from plastic toys into saliva, and thereby helping to ensure the safety of children.

REFERENCES

[1] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. Assoc Off Anal Chem Int, 86, (2003) 412.
[2] A. Preetha and R. Banerjee, Trends Biomater. Artif. Organs, 18 (2), (2005) 178.

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