



Determination of Phthalates Leached from Toys into Artificial Saliva

UCT Product #: ECPAHFR50CT (50 mL polypropylene centrifuge tubes)

ECQUUS2-MP (Mylar pouch with 4000 mg MgSO₄ and 2000 mg NaCl)

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Phthalates are plasticizers added to polymers to make them softer and more flexible. They are commonly used in the manufacturing of toys. Since phthalates are not permanently bonded in polymers, they may be released through touching, licking, or chewing. This new method artificially reproduces the licking and chewing actions of children by shaking cut toys in artificial saliva. Following extraction, phthalate levels are determined by GC/MS.

Preparation of Artificial Saliva (AS)¹

AS was prepared by mixing 0.18 g xanthan gum, 1.2 g potassium chloride, 0.85 g sodium chloride, 0.05 g magnesium chloride, 0.13 g calcium chloride, and 0.13 g di-potassium hydrogen orthophosphate with 1 L of reagent water (1) and stirred for 4 hours.

Procedure

1. Cut plastic toys into small pieces
2. Weigh 1 to 2 g of toy samples to a 50 mL centrifuge tube **ECPAHFR50CT**
3. Add 10 mL of **AS** and two stir bars to the 50 mL tube
4. Shake for 1 hour using a horizontal shaker
5. Transfer the **AS** to a new 50 mL tube, add 10 mL of ethyl acetate and shake for 1 min
6. Add salts from pouch **ECQUUS2-MP** and shake vigorously for 1 min
7. Centrifuge at 5000 rpm for 5 min
8. Transfer 1 mL of the supernatant into a 2 mL auto-sampler vial
9. Add 10 µL of 50 ppm triphenyl phosphate (TPP) as internal standard
10. The sample is ready for GC/MS analysis

INSTRUMENT CONDITIONS:

GC/MS: Agilent 6890N GC coupled with 5975C MSD, equipped with 7683 auto sampler

Injector: 1 µL splitless injection at 250 °C, 30 mL/min split vent at 1 min

Liner: 4 mm splitless gooseneck, 4mmID*6.5mmOD*78.5mm (UCT cat#: GCLGN4MM)

GC capillary column: Restek Rxi-5sil MS 30m*0.25mm*0.25µm integrated with 10m guard column

Oven temperature program: Initial temperature of 70 °C, hold for 1 min; ramp at 20 °C/min to 315 °C, hold for 4.75 min. Acquire data from 6 to 14 min.

Carrier gas: He at a constant flow of 1.2 mL/min.

MSD: Transfer line: 280 °C; MS Source (EI): 250 °C; MS Quad: 150 °C

Simultaneous Scan/SIM: Scan range: 55-350 amu

SIM Table

Compound	Abbreviation	Rt (min)	Group #	Start (min)	Dwell time (ms)	Quantify ion	Qualifier ion 1	Qualifier ion 2
Dimethyl phthalate	DMP	6.989	1	6	25	163	194	133
Diethyl phthalate	DEP	7.858	2	7.5	25	149	177	105
Dibutyl phthalate	DBP	9.865	3	9	25	149	223	150
Benzyl butyl phthalate	BBP	11.716	4	11	25	149	91	206
Triphenyl phosphate	TPP	11.964			25	326	325	
Bis(2-ethylhexyl) phthalate	DEHP	12.432	5	12.3	25	149	167	279
Di-n-octyl phthalate	DOP	13.138			25	149	279	150

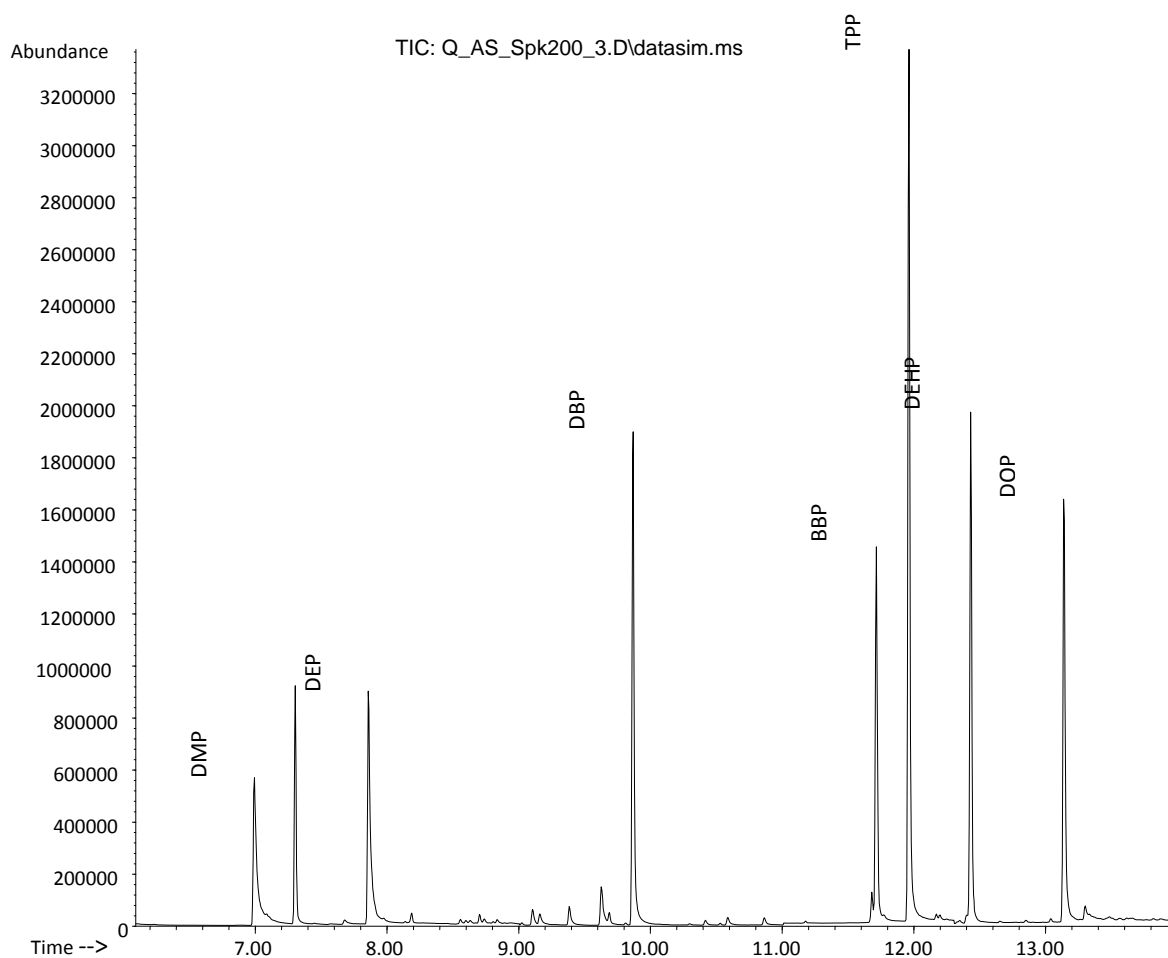
Calibration Curves

Matrix matched calibration curves are constructed by analyzing matrix matched standards (0-1000 ng/mL). The responses were linear with correlation coefficient higher than 0.9973. The limit of quantification of this method is 25 ng/mL.

Accuracy and Precision Data (n=4)*		
Analyte	Fortified 200 ng/mL	Fortified 500 ng/mL
	Recovery ± RSD %	Recovery ± RSD %
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

*The control sample did not contain phthalates

Chromatogram of AS fortified with 200 ng/mL phthalates



Results

Diethyl phthalate was found leaching from toy samples into artificial saliva at a concentration of 285 ng/g (RSD=5.9%, n=3).

References

- (1) A. Preetha and R. Banerjee, Comparison of Artificial Saliva Substitutes, *Trends Biomater. Artif. Organs* **18(2)**, 178-186 (2005).