Controlled Extraction & Simulation Study Results

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Introduction

For many parenteral drug products, the needed AET is below the limit of routine analytical science. The PODP Extractables and Leachables Chemistry Working Group has proposed the "Simulation Study" as a way to conduct meaningful analytical investigations at the desired limit.

A mock PODP was constructed using materials studied via CES (Controlled Extraction Study) using both organic and sealed vessel extractions. Three mock formulations were charged into these packages which were stored inverted for 6 months at 40 °C.

This presentation compares the CES and simulation study results.

Experimental

Test System consisted of
- LDPE Bottle (100 mL nominal fill volume), 14.9 g
- Polypropylene Cap, 3.1 g
- Adhesive label, 0.19 g
- Rubber gasket, 0.5 g

Each Test System component was extracted separately in water pH 2.5, water pH 9.5, hexane and isopropanol.
Three different simulated formulations were prepared:
- Water pH 2.5
- Water pH 9.5
- 50/50 Isopropanol/Water

Bottles were filled with simulated formulation (100 mL), sealed with gasket/caps, labeled, placed in foil multi-laminate overpouches, and stored inverted for 6 months at 40 °C. After storage, aliquots were removed for analytical testing.

Picture of Test Article Components

Samples were spiked with target leachable compounds to ensure species would be detectable. The following compounds were employed:

Compound	CAS	MW	Supplier
Methyl ethyl ketone (MEK)	78-93-3	72.1	Fluka
Irgacure 1173	7473-98-5	164.2	Sigma-Aldrich
Benzophenone	119-61-9	182.2	Sigma-Aldrich
Dipropylene glycol diacrylate (DGPTA)	57472-68-1	242.3	TCI Chemicals

Final spiking level in extract solution = 1.25 µg/mL for each

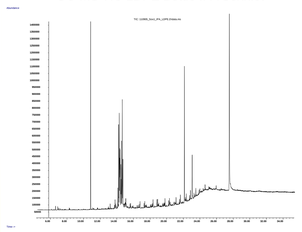
Additional Test Article Details

Material Type	Material Application	Material Format	Description
Low density polyethylene (LDPE)	Bottle/ Vial	Bottle	4 oz LDPE, part B347A (Container & Packaging Supply)
Polypropylene (PP)	Cap	Cap	PP, Part L764 (Container & Packaging Supply)
Adhesive Label	Label on Container Surface	Label Sheets	Substrate: Unknown Adhesive: Acrylic polymer(s), residual monomers, water, ammonia (99.55%); wetting agent, Surfynol 336, at 0.4% containing CAS 577-11-7 (> 25%), CAS 9014-85-1 (> 25%); Biocide, Kathon LX, at 0.05% containing Chloro-2-methyl-4-isothiazolin-3-one (CAS 26172-55-4), 1.1-1.4%,2-Methyl-4-isothiazolin-3-one (CAS 2682-20-4), 0.3 - 0.5%, Magnesium Chloride (CAS 7786-30-3), 1.0 - 1.2%, Magnesium nitrate (CAS 10377-60-3), 1.4 - 2.0% Copper nitrate (CAS 3251-23-8) 1,500 - 1,700 ppm, Water, 95 - 97% Printing ink: Irgacure 369 (CAS 119313-12-1) and Irgacure 1173 (CAS 7473-98-5), photoinitiators; Trimethylolpropane triacrylate (TMPTA, CAS 15625-89-5), Tripropylene glycol diacrylate (TPGDA, CAS 42978-66-5), Glycerol propoxy triacrylate (GPTA, CAS 52408-84-1), monomers; HQME/Mequinol (CAS 150-76-5), stabilizer; Carbon black (CAS 1333-86-4),Phthalo blue (CAS 147-14-8),Carbazole violet (CAS 215247-95-3), pigments Varnish : Unknown
Rubber (Elastomer) (RE)	Closures	Gasket/ liner	Brominated isobutylene isoprene copolymer (57.3%); calcined aluminum silicate, 38.2%, titanium dioxide, 1.2%; paraffinic oil, 1.2%; zinc oxide, 0.6%; polyethylene, 0.6%; SRF Carbon block mixture, 0.4%; calcined magnesium oxide, 0.3%; 4,4'-dithiodi-morpholine/polyisobutylene, 0.3%

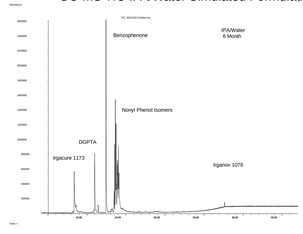
For analysis by GC-MS, each samples were extracted with an equivalent volume of methylene chloride and the organic layer analyzed directly. For analysis by HS GC-MS, samples were placed into sampling vials without pre treatment. For ICP-MS, aqueous samples were analyzed directly and for organic containing solvents, volatiles were evaporated prior to analysis.

Results

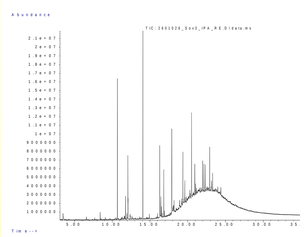
GC-MS TIC LDPE Bottle IPA Soxhlet



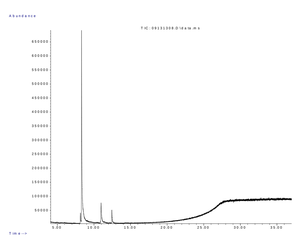
GC-MS TIC IPA/Water Simulated Formulation



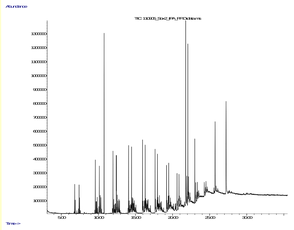
GC-MS TIC Bromobutyl Elastomer IPA Soxhlet



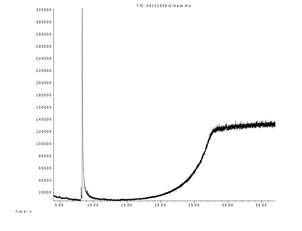
GC-MS TIC pH 2.5 Simulated Formulation



GC-MS TIC Polypropylene Cap IPA Soxhlet



GC-MS TIC pH 9.5 Simulated Formulation

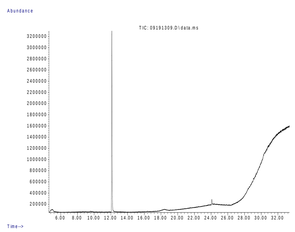


Headspace GC-MS Analysis of Packaging Components

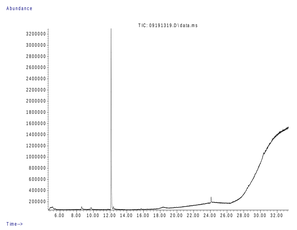
	Peak RRT	Proposed Substance	Attribute	µg/g (Elastomer: IS 1,4-Dioxane)
LDPE	0.248	Unknown	---	0.366
Elastomer	0.25	Unknown	---	1.2
Label	0.251	Unknown	---	8.7
LDPE	0.261	2-Methylpropene	Tentative	0.396
Label	0.282	Ethanal	Tentative	0.8
PP	0.341	Isopentane	Tentative	0.8
Label	0.4	Ethanol	Tentative	3.3
Label	0.463	Acetone	Tentative	7.2
Elastomer	0.465	Acetone	Tentative	0.7
LDPE	0.465	Acetone	Tentative	0.800
PP	0.538	2-Methylpentane	Tentative	38.0
PP	0.606	2-Methylpentene	Tentative	0.6
PP	0.622	Hexane	Tentative	3.2
Label	0.748	2-Propenoic acid, methyl ester	Tentative	20.6
PP	0.984	2,4-Dimethylhexane	Tentative	1.0
PP	1.083	3-Methylheptane	Tentative	0.8
PP	1.186	2,3,5-Trimethylhexane	Tentative	5.3
PP	1.196	2,4-Dimethylheptane	Tentative	66.6
PP	1.208	2,6-Dimethylheptane	Tentative	0.4
PP	1.242	Unknown		0.4
PP	1.249	2,4-Dimethyl-1-heptene	Tentative	2.4
PP	1.275	2,3-Dimethylheptane	Tentative	4.9
PP	1.285	4-Methyloctane	Tentative	27.0
LDPE	1.632	Unknown	---	0.282
PP	1.654	4-Methyl-1-undecene	Tentative	13.6
PP	1.664	Tetradecane	Tentative	4.8
PP	1.701	Unknown		1.0
PP	1.726	4,7-Dimethylundecane	Tentative	7.4
PP	1.735	5-Ethyl-2-methyloctane	Tentative	2.3
PP	1.746	4-Methylundecane	Tentative	0.8
Elastomer	1.902	Unknown	---	0.4
PP	2.001	Hydrocarbon related		1.4
PP	2.064	Hydrocarbon related		0.7

LDPE = LDPE Bottle
Elastomer = Bromobutyl Rubber Seal
Label = Affixed Label
PP = Polypropylene Cap

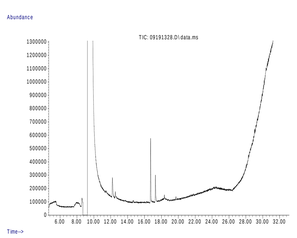
HS GC-MS TIC pH 2.5 Simulated Formulation



HS GC-MS TIC pH 9.5 Simulated Formulation



HS GC-MS TIC IPA/Water Simulated Formulation



Trace Element Comparison for CES of Elastomer to Simulation Study Results

Element	Extracted Amount in Elastomer, µg/g		Detected Amount in Simulant, µg/mL		
	pH 2.5 Extracts	pH 9.5 Extracts	PODP pH 2.5	PODP pH 9.5	PODP IPA/H_2O
Br	17.5	20.5	1.100	0.190	2.950
K	ME[1]	6.84	ME[1]	0.360	0.009
Ca	4.07	2.07[2]	0.120	0.015	0.145
Na	3.05	ME[1]	1.100	ME[1]	1.675
Mg	3.50	2.90	0.120	0.024	0.223
Al	0.66	3.56	0.071	0.034	0.010
Zn	2.89	0.49	0.140	0.003	0.020
Fe	0.33	0.08[2]	0.009	0.000	0.008
Ti	0.29	NP[3]	0.000	0.520	0.001
Si	0.10	0.25	0.070	0.069	0.055
Ni	0.01	0.01	0.002	0.001	0.000
V	0.01	0.01	NP[3]	NP[3]	NP[3]
Cr	0.01	0.01[2]	0.001	0.001	0.000
Mn	0.01	NP[3]	0.001	0.000	0.000
Sr	0.01	0.01	0.001	0.000	0.001

Notes:
[1]ME = this element a component of the extracting solution used and thus was not measurable as an extractable.
[2]Detected in only one of the two replicate extracts.
[3]NP = not present in this extract in measurable quantities.

CES Instrumental Methods

Headspace GC-MS

Headspace:	Leap Technologies CombiPAL
GC-MS:	Agilent 6890/5973
Injection Mode:	Split
Injector Temperature:	240 °C
Column:	Restek Rtx-624, 60 m x 0.32 mm ID, 1.8 µm film thickness
Split Flow	5.0 mL/min
Split Ratio:	5:1
Oven Temperature:	35 °C for 5 minutes, heat at 8°C/minute to 190 °C, hold at 190 °C for 2 minutes, heat at 25°C/minute to 240 °C, hold at 240 °C for 5 minutes
Pressure Program:	Constant flow (Helium) at 1.0 mL/minute
Transfer Line Temp:	240 °C
Ionization Mode:	Electron Ionization
Scan Mode:	Scanning; m/z 30-650
Incubation temp:	90 °C
Incubation time:	60 minutes
Injection volume:	1 mL
Agitation on	3 sec
Agitation off	2 sec
Agitation speed	500 rpm

ICP-MS

ICP-MS	Agilent 7500A
Forward Power	1300 watts
Integration Time	0.1 seconds per point
Rinse Time	180 seconds
Rinse Rate	0.5 rps
Uptake Time	45 seconds
Uptake Rate	0.5 rps
Stabilization Time	20 Seconds
Analysis Pump Rate	0.1 rps
All Other Settings	Determined by Tune

The following metals/elements were tested: Li, Be, B, Na, Mg, Al, Si, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, I, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Th, and U

GC-MS (Direct Inject)

GC-MS	Agilent 6890/5973
Injection Mode:	Splitless
Injection Volume:	1.00 µL
Injector Temperature:	280 °C
Column:	Restek Rtx-1 30 m x 0.25 mm ID x 0.1 µm film thickness
Oven Temperature:	40 °C for 1 minute, heat at 10 °C/minute to 300 °C, hold at 300 °C for 10 minutes
Flow Program:	Constant flow (Helium) at 1.00 mL/minute
Purge Flow:	60.0 mL/minute, on at 1.00 minutes
Transfer Line:	280 °C
Ionization Mode:	Electron Ionization
Scan Mode:	Scanning; m/z 50-650
MS Quad Temp:	150 °C
MS Source Temp:	230 °C

Simulation Study Instrumental Methods

Headspace GC-MS

Headspace:	Pelkin Elmer Headspace Sampler
GC-MS:	Agilent 6890/5973
Injection Mode:	Split
Injector Temperature:	125 °C
Column:	Agilent DB-624 60 m x 0.32 mm ID, 1.8 µm film thickness
Split Flow	5.0 mL/min
Split Ratio:	1:1
Oven Temperature:	40 °C for 4 minutes, heat at 12 °C/minute to 230 °C, hold at 230 °C for 17 minutes
Pressure Program:	Constant Pressure (Helium) 200 kPa
Transfer Line Temp:	280 °C
Ionization Mode:	Electron Ionization
Scan Mode:	Scanning; m/z 29-500

ICP-MS

ICP-MS	Agilent 7500A
Forward Power	1300 watts
Integration Time	0.1 seconds per point
Rinse Time	180 seconds
Rinse Rate	0.5 rps
Uptake Time	45 seconds
Uptake Rate	0.5 rps
Stabilization Time	20 Seconds
Analysis Pump Rate	0.1 rps
All Other Settings	Determined by Tune

The following metals/elements were tested: Li, Be, B, Na, Mg, Al, Si, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, I, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Th, and U

GC-MS (Direct Inject)

GC-MS	Agilent 7890/5973C
Injection Mode:	Splitless
Injection Volume:	1.00 µL
Injector Temperature:	300 °C
Column:	Agilent DB 5ms, 30 m, 0.25mm ID x 0.25 film thickness
Oven Temperature:	50 °C for 1 minute, heat at 12 °C/minute to 325 °C, hold at 325 °C for 16 minutes
Flow Program:	Constant flow (Helium) at 1.2 mL/minute
Purge Flow:	60.0 mL/minute, on at 1.00 minutes
Transfer Line:	280 °C
Ionization Mode:	Electron Ionization
Scan Mode:	Scanning; m/z 35-650
MS Quad Temp:	150 °C
MS Source Temp:	230 °C

Discussion

Shown are representative profiles from Soxhlet extraction of elastomer, LDPE bottle and polypropylene cap components. Many obvious extractables can be seen without close examination of instrumental baseline. By comparison, the next set of three vertical profiles from the three simulation study formulations are markedly simpler except for the lipophilic simulant 50/50 IPA/H_2O. In that formulation, the obvious envelope of nonylphenol isomers can be correlated in a qualitative sense to the LDPE bottle. The largest single peaks in that simulant are the spiked compounds used as a positive control for label migration over the course of the study. Even after 6 months at elevated temperatures, all label components dominate the simulant profiles.

Compared to how the late eluting antioxidant (Irganox 1076) dominates the IPA Soxhlet extract from the bottle, it is a very minor component of the 50/50 IPA/H_2O simulant. But it is still observed. The other major aliphatic components from the elastomer as well as from the bottle cannot be seen in any of the profiles.

The table of headspace identified targets from the packaging component are also dominated by aliphatic hydrocarbons which cannot be observed in the three horizontal profiles of simulation formulation. The two aqueous simulation formulations only show the spiked target compounds while the 50/50 IPA/H_2O solution show two small peaks attributed to butanol and methyl isobutyl ketone. Neither of these peaks were observed in any of the extracts during the initial identification exercise. These may be either reaction products or minor constituents of the spiking solution.

Initial analysis of elemental impurities arising from the bottle showed very little present in sealed vessel extractions at either low or high pH. By comparison, the elastomer showed more such targets with elemental bromine being the largest constituent. Unfortunately, the reported amounts are semi-quantitative and were not found to provide adequate mass balance between components and simulation formulation.

Overall, the comparison of the simulation study to the CES shows qualitatively fewer targets. Further work to investigate whether polar extractables as detected by HPLC follow similar behavior. These data were collected during the course of this study, but are not presented here.

Conclusions

Quantities and types of organic extractables appear to be less in simulated formulation than found in the CES. Quantitative methods to provide more accurate comparisons between the purported source of the targets found in the simulation study and the targets found in the CES will need to be assessed more closely, especially for elemental impurities.

Acknowledgments

The authors would like to acknowledge the collaborative efforts of all members of the Toxicology and Chemistry sub-teams of the PQRI PODP working group. All research work is supported under the direction of PQRI.