Comprehensive Extractables Analysis of Medical Grade O-Ring

Dan Ewing,[1] Bill Hurley,[2] Andrew Feilden,[3] Michael Creese,[3] Kate Comstock,[4] and John Schmelzel[4]
[1]Parker Hannifin O-Ring Division, Lexington, KY, USA; [2]Darcoid Nor-Cal Seal, Oakland, CA;
[3]Simthers Rapra, Shrewsbury, UK; [4]Thermo Fisher Scientific, San Jose, CA, USA

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Introduction

USP class VI materials are commonly used in medical devices, biopharmaceutical manufacturing process equipment, pharmaceutical packaging, and direct patient-contact applications. For these intended uses, class VI materials need to undergo extractables testing to meet regulatory requirements.

In this study, comprehensive extractables analysis of USP class VI O-rings was conducted using Thermo Scientific™ Q Exactive™ Plus MS and Thermo Scientific™ iCAP™ Q ICP-MS for trace element analysis. Thermo Scientific™ software SIEVE™, Mass Frontier™, and mzCloud™ spectral database were used for data processing.

LCMS Analyses

Sample Preparation

Four different types of medical grade O-rings, A, B, C, and D, were extracted using both water for injection (WFI) and IPA by reflux extraction. For each extraction, 5g of O-ring material and 50 ml of solvent were refluxed for 4 hours. The extracts solutions were analyzed directly by LCMS.

Liquid Chromatography

LC separations were carried out on the Thermo Scientific™ Ultimate™ 3000 RS UHPLC system consisting of: DGP-3000RS pump, WPS-3000RS sampler, TCC-3000RS column compartment and DAD-3000RS UV detector.

Column: Thermo Scientific™ Hypersil™ C18, 2.1x150 mm 1.9 µl Column Temp: 50 °C

LC Mobile phase: A: H_2O/0.1% formic acid B: ACN/0.1% Formic acid

Gradient:

Time (min)	0	3	35	40	40.1	45
B%	10	20	90	90	10	10

Flow rate (µl/min): 400 Injection volume (µl): 10

Mass Spectrometry

MS analyses were carried out on the Thermo Scientific™ Q Exactive™ Plus mass spectrometer using electrospray ionization (ESI) and external calibration. High resolution full scan MS and data dependant top 3 MS/MS data were collected with polarity switching. The resolving power was 70,000 and 17,500 (FWHM m/z200) respectively for full scan and ms/ms . The scan range is 125-1700 amu. HCD stepped normalized collision energy was 25, 35, and 45.

Thermo Scientific™ Q Exactive™ Plus mass spectrometer with a Thermo Scientifc™ UltiMate™ 3000 RS UHPLC system

Source Conditions:
Ionization mode: ESI pos/neg switching
Sheath gas flow rate: 50
Auxiliary gas flow rate: 10
Spray voltage (KV): (+) 3.5 pos / 2.5(-) neg
Capillary temp (°C): 300
S-lens RF level: 55.0
Probe heater temp (°C): 430

Q Exactive Plus MS HRAM Data Acquisition

The High Resolution Accurate Mass (HRAM) data acquisition on Q Exactive Plus MS using full scan/top3 ms/ms with polarity switching generated high quality data, ensuring the detection of structurally diversified compounds at all levels. Figure 1 shows high mass accuracy for pos/neg switching on the fly and the fine isotope pattern of A2. The Δmass1.9954 between A0 and A2 indicate one sulfur present.

FIGURE 1. High Mass Accurate and Fine Isotope Pattern for Pos/Neg Switching

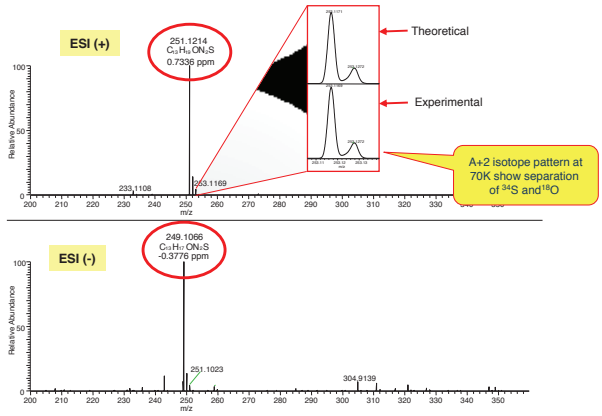

FIGURE 2. Base Peak Chromatographs of Full Scan WFI Extraction (ESI+)

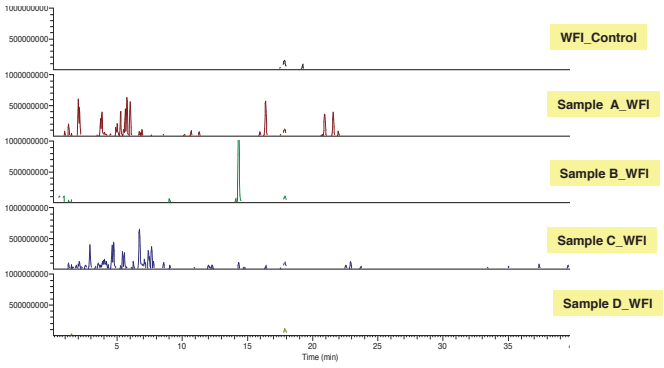

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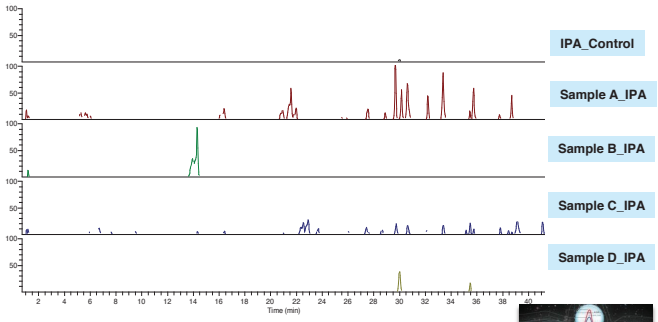
FIGURE 3. Base Peak Chromatographs of Full Scan IPA Extraction (ESI+)



Data Analysis

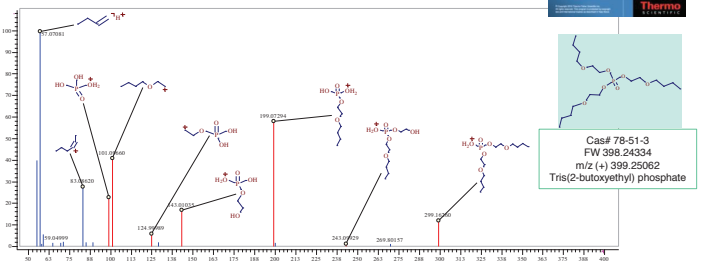
SIEVE software for Component Extraction and Database Search

The data was processed using SIEVE 2.2 software for component extraction and differential analysis. The extracted components were filtered using the versatile and customizable filters and then searched against ChemSpider™ database for structure identification .

Component Identification and Structure Elucidation

ChemSpider database searching generated multiple structures for each extracted components. HRAM data with 3 ppm threshold setting reduced the possible structures. To determine the correct structure(s), ms/ms fragments library search was carried out using "Mass Frontier" software, a small molecule structure analysis software. The "HighChem Fragmentation Library" in Mass Frontier software 7.0 has extensive published literature references. For each proposed structure, the "Fragments and Mechanisms" feature in Mass Frontier software was used to generate predicted "fragments and mechanisms" through HighChem Fragmentation Library search, see figure 4. A high degree of correlation between predicted and experimental fragments confirms the proposed structure. Mass Frontier software then automatically annotates the matching fragments based on library search results.

Figure 4. Fragment and Mechanism for Structure Elucidation



mzCloud High Resolution Spectral Database Searching

A search was conducted with "mzCloud". Figure 5 A and B show identification using the ms/ms spectrum search feature.

mzCloud™ is a freely searchable high resolution spectral database at www.mzcloud.org. mzCLoud provides several search criteria for small molecule structure identification using tandem mass spectra, including spectra, fragments, precursor ions, etc, all of which can be very useful for unknown structure elucidation.

FIGURE 5-A. Copy MS/MS Spectrum to mzCloud for Substructure Identification

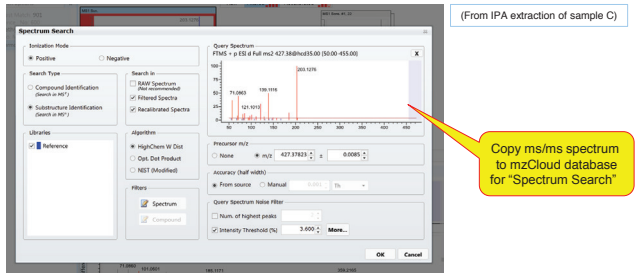


FIGURE 5-B. Spectrum Search Result for Bis (2-ethylhexyl) sebacate

FIGURE 6. Base Peak Chromatogram of WFI Extraction of Sample A

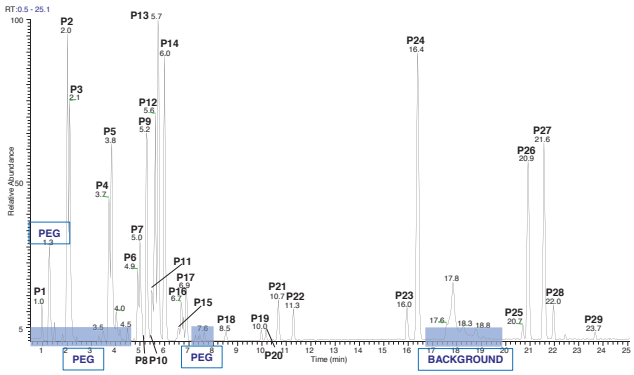
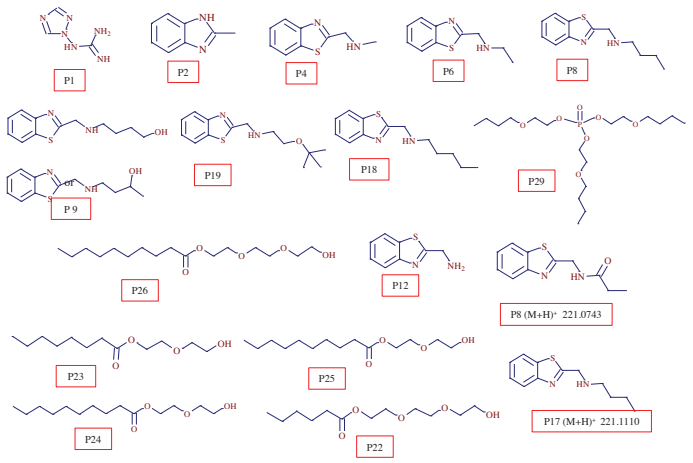


TABLE 1. Components Identified from WFI Reflux of Sample-A (Partial List)

Peak ID	RT	Measured (M+H)+	Calculated (M+H)+	Elemental Composition	Error (PPM)
1	1.0	127.0733	127.0723	C3H6N6	
2	2.0	133.0763	133.0761	C8H8N2	2.1
3	2.1	133.0763	133.0761	C8H8N2	2.1
4	3.7	179.0639	179.0638	C9H10N2S	0.8
5	3.8	179.0639	179.0638	C9H10N2S	0.8
6	4.9	193.0798	193.0794	C10H12N2S	2
7	5.0	193.0798	193.0794	C10H12N2S	1.9
8	5.1	221.0747	221.0743	C11H12ON2S	1.6
9	5.2	237.1058	237.1056	C12H16ON2S	0.7
10	5.3	251.1214	251.1213	C13H18ON2S	0.6
11	5.5	149.0713	149.0709	C8H8ON2	2.1
12	5.6	165.0484	165.0481	C8H8N2S1	1.9
13	5.7	165.0484	165.0481	C8H8N2S1	1.9
14	6.0	251.1214	251.1213	C13H18ON2S	0.7
15	6.6	251.1218	251.1213	C13H18ON2S	1.5
16	6.7	163.1331	163.1329	C8H18O3	0.97
17	6.9	221.1110	221.1107	C12H16N2S	1.3
18	8.5	235.1267	235.1264	C13H18N2S	1.6
19	10.0	265.1372	265.1369	C14H20ON2S	1.1
20	10.2	265.1372	265.1369	C14H20ON2S	1.2
21	10.7	219.1958	219.1955	C12H26O3	1.5
22	11.3	249.1698	249.1697	C12H24O5	1
23	16.0	233.1751	233.1747	C12H25O4	1.5
24	16.4	277.2011	277.2010	C14H28O5	0.2
25	20.7	261.2064	261.2060	C14H28O4	0.4
26	20.9	305.2324	305.2324	C16H32O5	0.1
27	21.6	325.1433	325.1434	C20H20O4	0.1
28	22.0	325.1438	325.1434	C20H20O4	0.3
29	23.7	399.2511	399.2506	C18H40O7P	1.4

TABLE 2. Proposed Structures for WFI Reflex of Sample A (Partial List)



ICPMS Analyses

The samples were prepared by placing the O-rings in 25 ml DI water and 25 ml 2% nitric acid and soaked at RT for 24 hours. The analyses were conducted on Thermo Scientific iCAP Q ICP-MS with He KED (Kinetic Energy Discrimination) interference reduction mode setting. The instrument was standardized at 10 ppb.

To determine if trace and potentially toxic metals were leached from the O-rings, the USP<232> Class1 & 2 elements and additional elements which are commonly analyzed by ICP-MS were determined.

The analyses results for the four types of O-rings showed that they are clean of all Class 1 & 2 elements, see Figure 7 for the ICPMS results for sample 1A and B.

The system control software Qtegra provides full 21CFR Part 11 tool set to operate under compliant environments.

FIGURE 7. ICPMS Results for O-Rings Sample A and B (C & D not shown)

	DI blank	DI EPDM	Nitric Blank	Nitric EPDM	IDL	PQL
66Zn (KED)	0.533	241.029	nd	684.411	0.060	0.599
23Na (KED)	1.177	81.969	nd	113.758	0.172	1.724
44Ca (KED)	0.093	48.462	nd	89.881	0.058	0.578
39K (KED)	nd	64.572	nd	85.289	0.368	3.683
24Mg (KED)	nd	18.399	nd	83.517	0.029	0.288
27Al (KED)	nd	nd	nd	48.833	0.090	0.903
56Fe (KED)	nd	nd	nd	38.912	0.015	0.147
31P (KED)	nd	3.377	nd	8.442	1.169	11.688
63Cu (KED)	nd	0.789	0.104	7.142	0.004	0.042
137Ba (KED)	nd	2.484	nd	5.240	0.004	0.045
55Mn (KED)	nd	0.963	nd	3.893	0.004	0.045
88Sr (KED)	nd	1.340	nd	2.620	0.004	0.035
52Cr (KED)	nd	0.027	nd	1.076	0.001	0.009
60Ni (KED)	nd	0.127	nd	1.052	0.003	0.030
48Ti (KED)	nd	0.303	nd	0.897	0.021	0.213
208Pb (KED)	nd	nd	nd	0.304	<0.001	<0.01
59Co (KED)	nd	0.012	0.001	0.200	<0.001	<0.01
209Bi (KED)	nd	nd	nd	0.174	<0.001	<0.01
118Sn (KED)	nd	nd	nd	0.159	0.006	0.056
140Ce (KED)	nd	nd	nd	0.101	<0.001	<0.01
85Rb (KED)	nd	0.049	nd	nd	0.004	0.039
121Sb (KED)	nd	0.008	nd	0.044	0.004	0.041
95Mo (KED)	nd	nd	nd	0.036	0.001	0.014
90Zr (KED)	nd	nd	nd	0.034	0.002	0.016
45Sc (KED)	nd	nd	nd	nd	0.004	0.042
7Li (KED)	nd	nd	nd	nd	0.074	0.744
9Be (KED)	nd	nd	nd	nd	0.045	0.458
11B (KED)	nd	nd	nd	nd	0.423	4.226
51V (KED)	nd	nd	nd	nd	0.025	0.255
71Ga (KED)	nd	nd	nd	nd	0.001	0.011
73Ge (KED)	nd	nd	nd	nd	0.008	0.083
75As (KED)	nd	nd	nd	nd	0.007	0.072
78Se (KED)	nd	nd	nd	nd	0.022	0.223
89Y (KED)	nd	nd	nd	nd	0.002	0.019
93Nb (KED)	nd	nd	nd	nd	0.001	0.007
101Ru (KED)	nd	nd	nd	nd	<0.001	<0.01
103Rh (KED)	nd	nd	nd	nd	<0.001	<0.01
105Pd (KED)	nd	nd	nd	nd	<0.001	<0.01
107Ag (KED)	nd	nd	nd	nd	<0.001	<0.01
111Cd (KED)	nd	nd	nd	nd	0.002	0.015
115In (KED)	nd	nd	nd	nd	<0.001	<0.01
125Te (KED)	nd	nd	nd	nd	<0.001	<0.01
133Cs (KED)	nd	nd	nd	nd	<0.001	<0.01
139La (KED)	nd	nd	nd	nd	<0.001	<0.01
141Pr (KED)	nd	nd	nd	nd	<0.001	<0.01
146nd (KED)	nd	nd	nd	nd	<0.001	<0.01
147Sm (KED)	nd	nd	nd	nd	<0.001	<0.01
153Eu (KED)	nd	nd	nd	nd	<0.001	<0.01
157Gd (KED)	nd	nd	nd	nd	<0.001	<0.01
159Tb (KED)	nd	nd	nd	nd	<0.001	<0.01
163Dy (KED)	nd	nd	nd	nd	<0.001	<0.01
165Ho (KED)	nd	nd	nd	nd	<0.001	<0.01
166Er (KED)	nd	nd	nd	nd	<0.001	<0.01
169Tm (KED)	nd	nd	nd	nd	<0.001	<0.01
172Yb (KED)	nd	nd	nd	nd	<0.001	<0.01
175Lu (KED)	nd	nd	nd	nd	<0.001	<0.01
178Hf (KED)	nd	nd	nd	nd	0.001	0.007
181Ta (KED)	nd	nd	nd	nd	<0.001	<0.01
182W (KED)	nd	nd	nd	nd	0.001	0.007
185Re (KED)	nd	nd	nd	nd	<0.001	<0.01
189Os (KED)	nd	nd	nd	nd	<0.001	<0.01
193Ir (KED)	nd	nd	nd	nd	<0.001	<0.01
195Pt (KED)	nd	nd	nd	nd	<0.001	<0.01
202Hg (KED)	nd	nd	nd	nd	<0.001	<0.01
205Tl (KED)	nd	nd	nd	nd	<0.001	<0.01
232Th (KED)	nd	nd	nd	nd	<0.001	<0.01
238U (KED)	nd	nd	nd	nd	<0.001	<0.01

	DI blank	DI Perfluoroelast	Nitric Blank	Nitric Perfluoin	IDL	PQL
39K (KED)	nd	25.958	0.998	41.121	0.368	3.683
23Na (KED)	1.177	34.602	nd	36.954	0.172	1.724
31P (KED)	nd	4.915	nd	35.975	1.169	11.688
27Al (KED)	nd	1.642	0.408	14.075	0.090	0.903
48Ti (KED)	nd	1.006	nd	12.762	0.021	0.213
56Fe (KED)	nd	0.314	nd	10.720	0.015	0.147
24Mg (KED)	nd	2.728	nd	7.218	0.029	0.288
66Zn (KED)	0.533	11.534	nd	6.269	0.060	0.599
44Ca (KED)	0.093	3.146	nd	5.385	0.058	0.578
137Ba (KED)	nd	45.947	nd	1.599	0.004	0.045
63Cu (KED)	nd	1.996	0.104	1.549	0.004	0.042
60Ni (KED)	nd	1.103	nd	1.090	0.003	0.030
52Cr (KED)	nd	1.501	nd	1.080	0.001	0.009
95Mo (KED)	nd	nd	nd	0.240	0.001	0.014
55Mn (KED)	nd	0.393	nd	0.234	0.004	0.045
90Zr (KED)	nd	nd	nd	0.231	0.002	0.016
88Sr (KED)	nd	0.494	nd	0.204	0.004	0.035
118Sn (KED)	nd	0.071	nd	0.189	0.006	0.056
59Co (KED)	nd	1.093	nd	0.132	<0.001	<0.01
208Pb (KED)	nd	0.040	nd	0.087	<0.001	<0.01
121Sb (KED)	nd	0.246	nd	nd	0.004	0.041
209Bi (KED)	nd	nd	nd	0.021	<0.001	<0.01
7Li (KED)	nd	nd	nd	nd	0.074	0.744
9Be (KED)	nd	nd	nd	nd	0.046	0.458
11B (KED)	nd	nd	nd	nd	0.423	4.226
45Sc (KED)	nd	nd	nd	nd	0.004	0.042
51V (KED)	nd	nd	nd	nd	0.025	0.255
71Ga (KED)	nd	nd	nd	nd	0.001	0.011
73Ge (KED)	nd	nd	nd	nd	0.008	0.083
75As (KED)	nd	nd	nd	nd	0.007	0.072
78Se (KED)	nd	nd	nd	nd	0.022	0.223
85Rb (KED)	nd	nd	nd	nd	0.004	0.039
89Y (KED)	nd	nd	nd	nd	0.002	0.019
93Nb (KED)	nd	nd	nd	nd	0.001	0.007
101Ru (KED)	nd	nd	nd	nd	<0.001	<0.01
103Rh (KED)	nd	nd	nd	nd	<0.001	<0.01
105Pd (KED)	nd	nd	nd	nd	<0.001	<0.01
107Ag (KED)	nd	nd	nd	nd	<0.001	<0.01
111Cd (KED)	nd	nd	nd	nd	0.002	0.015
115In (KED)	nd	nd	nd	nd	<0.001	<0.01
125Te (KED)	nd	nd	nd	nd	<0.001	<0.01
133Cs (KED)	nd	nd	nd	nd	<0.001	<0.01
139La (KED)	nd	nd	nd	nd	<0.001	<0.01
140Ce (KED)	nd	nd	nd	nd	<0.001	<0.01
141Pr (KED)	nd	nd	nd	nd	<0.001	<0.01
146nd (KED)	nd	nd				
147Sm (KED)	nd	nd				
153Eu (KED)	nd	nd				
157Gd (KED)	nd	nd				
159Tb (KED)	nd	nd				
163Dy (KED)	nd	nd				
165Ho (KED)	nd	nd				
166Er (KED)	nd	nd				
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172Yb (KED)	nd	nd				
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178Hf (KED)	nd	nd				
181Ta (KED)	nd	nd				
182W (KED)	nd	nd				
185Re (KED)	nd	nd				
189Os (KED)	nd	nd				
193Ir (KED)	nd	nd				
195Pt (KED)	nd	nd				
202Hg (KED)	nd	nd				
205Tl (KED)	nd	nd				
232Th (KED)	nd	nd				
238U (KED)	nd	nd				

Thermo Scientific™ iCAP™ Q ICP-MS

Conclusion

This study demonstrated a workflow for rubber O-ring extractable analysis using HRAM data acquisition, data-process software, and database search.

The UHPLC/ HRAM full MS/HCD MS^2 with polarity switching data acquisition on Q Exactive Plus MS, coupled with effective process software and database search using SIEVE, Mass Frontier, and mzCloud, significantly increased the confidence and throughput of routine extractable analysis, particularly for unknown components identification and structure characterization.

WFI and IPA extraction profiles of the four types of medical grade O-rings were quickly established by using this workflow. Data not show for sample B, C, and D.

The ICPMS results show that the four samples have none or very low level trace element present. GCMS analysis was carried out but the data has not been reported.

References

1. ISO10993-5 and -10.
2. USP <1663>
3. PQRI "L/E Recommendations to the FDA" http://www.pqri.org/publications/index.asp

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