

# Comprehensive Analysis of Extractables from Rubber Stopper used in Medical Devices and Pharmaceutical Products

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# Introduction

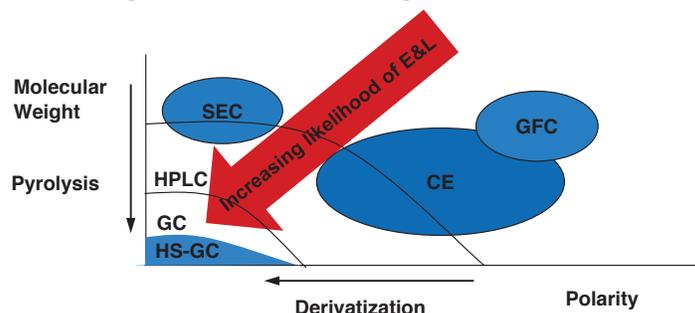
Rubbers & plastics are widely used in medical & drug delivery devices and packaging materials. Extractables & leachables assessment of all materials, especially from elastomeric & oligomeric components, forms an integral part of the submission for approval of a new drug product or medical device [1].

**Extractable** = possible impact. Test the material

**Leachable** = actual impact. Test the product

The mass spectrometer plays an important role in E&L identification and structure elucidation, as it is coupled with many techniques for definitive analysis, see figure 1. Here we present a comprehensive workflow for medical grade rubber stopper extractable analysis using multiple techniques including HR-LCMS, GCMS, and ICPMS, followed by data processes using novel software and database searching.

**FIGURE 1. Potential analytical techniques with increasing chance of extractables and leachables being found as the molecular weight decreases.**



## LCMS Analyses

### Sample Preparation

Four different types of medical grade rubber stoppers, sample-A, sample-B, sample-C, and sample-D, from Qure Medical, were extracted using DI water and IPA utilizing reflux extraction and a pressurized liquid extraction system. The extracts solutions were analyzed directly by LCMS.

### Liquid Chromatography

LC separations were carried out on the Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC 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 μm  
Column Temp: 50°C  
LC Mobile phase: A: H<sub>2</sub>O B: MeOH C: 50 mM Ammonium Acetate

### Mass Spectrometry

MS analyses were carried out on the Thermo Scientific™ Q Exactive™ mass spectrometer using both electrospray ionization (ESI) and atmosphere pressure chemical ionization (APCI). High resolution full scan MS and top 3 MS/MS data were collected in a data-dependent fashion at a resolving power of 70,000 and 17,500 (FWHM m/z 200) with polarity switching. The scan range is m/z150-1500. Stepped NCE (Normalized Collision Energy) setting was: 30, 40, 50.

### LCMS Result

The all-in-one full scan/top3 ms/ms with polarity switching data acquisition using both ESI, APCI ionization ensures the detection of structurally diversified compounds. It provides comprehensive extractable profiles of the rubber stoppers, see Figures 2 and 3.

**FIGURE 2. LCMS Chromatogram of IPA Reflux of Sample A**

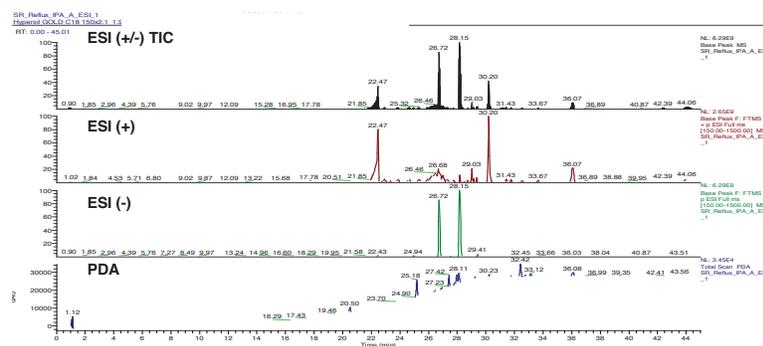
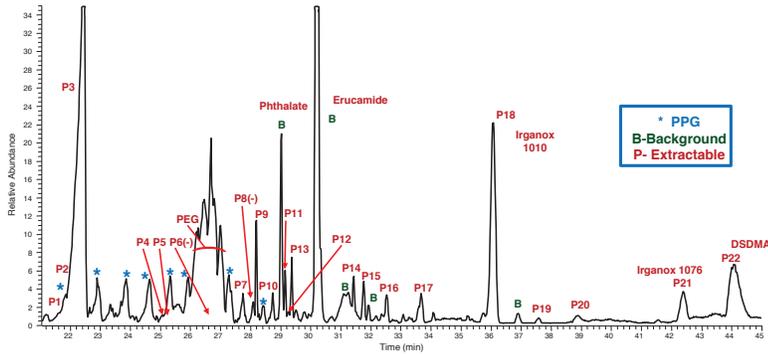


FIGURE 3. MS Chromatogram of IPA Reflux of Sample-A (ESI+)

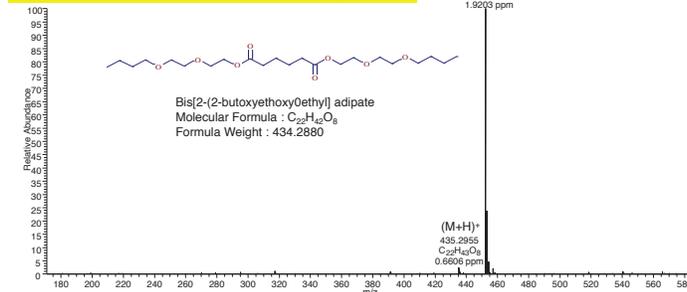


Component Detection and Structure Elucidation

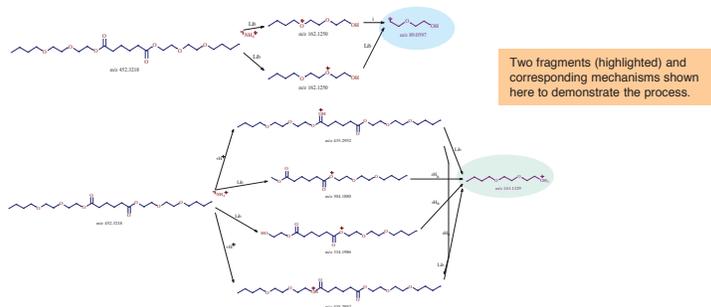
The High Resolution Accurate Mass (HRAM) data were processed using differential analysis software SIEVE™ 2.1 for component extraction. ChemSpider database searching was carried out to obtain possible structures of extracted components. While many possible hits were obtained for each component, to determine the correct structures, “Thermo Scientific™ Mass Frontier™ Software”, a small molecule structure analysis software, was used. The “HighChem Fragmentation Library™” in Mass Frontier 7.0 has extensive published literature references. For each proposed structure, the “Fragments and Mechanisms” feature in Mass Frontier was used to generate predicted “fragments and mechanisms” through HighChem Library search, see figure 4-b. A high degree of correlation between predicted and experimental fragments (indicated in red, see figure 4-c) confirms the proposed structure. Mass Frontier then automatically annotates the matching fragments based on library search results, see figure 4-c.

FIGURE 4. HR Full Scan and HCD MS<sup>2</sup> for Component ID and Structure Elucidation

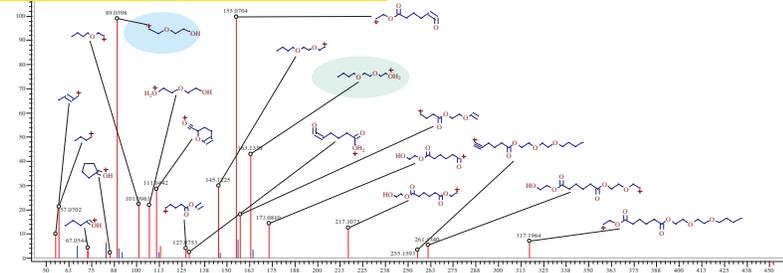
4-a. HRAM Full MS to Determine Elemental Composition



4-b. HighChem Fragmentation Library Search to Predict Fragments and Mechanism



4-c. HRAM MS/MS Spectra For Structure Elucidation



## mzCloud Spectral Database Searching



A search was also conducted against Thermo Scientific™ mzCloud™ Library, a newly developed high resolution spectral database. mzCloud library 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 shows identification of Irganox 1010 using the ms/ms spectrum search feature. The accuracy of searching result is indicated by matching score between the query and library spectra.

FIGURE 5. mzCloud library Search Results for Irganox 1010

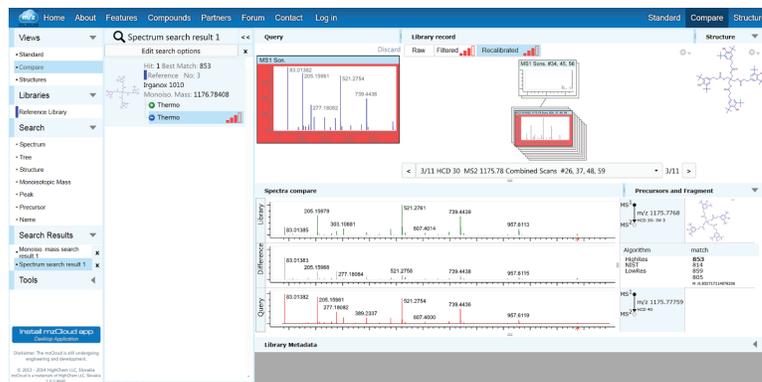
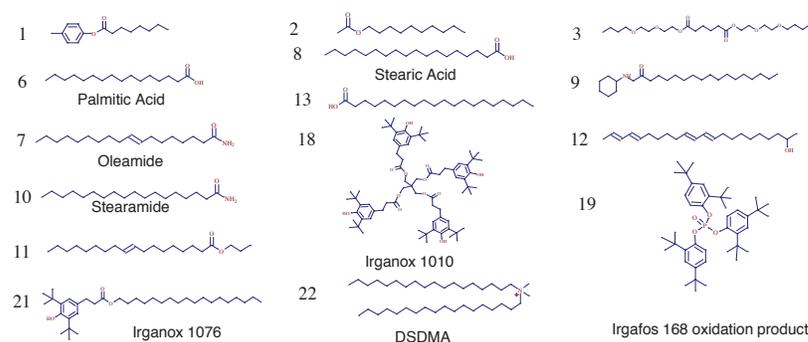


TABLE 1. Components Identified from IPA Reflux of Sample-A (Partial List)

Peak ID	RT	Mode	Measured (M+H) <sup>+</sup>	Calculated (M+H) <sup>+</sup>	Elemental Composition	Error (ppm)
1	21.48	APCI (+)	235.1691	235.1693	C <sub>15</sub> H <sub>22</sub> O <sub>2</sub>	-0.67
2	22.43	ESI (-)	199.1707 (M-H) <sup>-</sup>	199.1693 (M-H) <sup>-</sup>	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	2.0
3	22.47	ESI (+)	435.2956 (M+NH <sub>4</sub> ) <sup>+</sup>	435.2952	C <sub>22</sub> H <sub>42</sub> O <sub>8</sub>	0.86
4	24.94	ESI (-)	227.2017 (M-H) <sup>-</sup>	227.2006 (M-H) <sup>-</sup>	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	2.23
5	24.87	ESI (+)	219.1743	219.1743	C <sub>15</sub> H <sub>22</sub> O	1.8
6	26.72	ESI (-)	255.2330 (M-H) <sup>-</sup>	255.2391 (M-H) <sup>-</sup>	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	3.5
7	27.75	ESI (+)	282.2791	282.2791	C <sub>18</sub> H <sub>36</sub> ON	-0.11
8	28.15	ESI (-)	283.2643 (M-H) <sup>-</sup>	283.2632 (M-H) <sup>-</sup>	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	2.8
9	28.19	ESI (+)	383.3396 (M+NH <sub>4</sub> ) <sup>+</sup>	383.3396 (M+NH <sub>4</sub> ) <sup>+</sup>	(C <sub>24</sub> H <sub>47</sub> ON)+NH <sub>4</sub>	0.42
10	28.71	ESI (+)	284.2946	284.2948	C <sub>18</sub> H <sub>37</sub> O <sub>1</sub> N <sub>1</sub>	-0.5
11	29.14	ESI (+)	325.3097	325.3101	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	1.3
12	29.36	ESI (+)	319.2992	319.2995	C <sub>22</sub> H <sub>38</sub> O <sub>1</sub>	-1.1
13	29.41	ESI (-)	311.2963 (M-H) <sup>-</sup>	311.2945 (M-H) <sup>-</sup>	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	2.4
14	31.43	ESI (+)	340.3568	340.3574	C <sub>22</sub> H <sub>46</sub> ON	-1.8
15	31.77	ESI (+)	366.3729	366.3730	C <sub>24</sub> H <sub>48</sub> ON	-0.5
16	32.52	ESI (+)	409.3100	409.3101	C <sub>28</sub> H <sub>40</sub> O <sub>2</sub>	-0.34
17	33.70	ESI (+)	1227.00	1227.00	?	
18	36.00	ESI (+)	1194.8170 (M+NH <sub>4</sub> ) <sup>+</sup>	1194.8179 (M+NH <sub>4</sub> ) <sup>+</sup>	C <sub>73</sub> H <sub>108</sub> O <sub>12</sub>	-0.3
19	37.57	ESI (+)	663.4536	663.4537	C <sub>42</sub> H <sub>63</sub> O <sub>4</sub> P	-0.19
20	38.85	ESI (+)	522.5969	522.5972	C <sub>36</sub> H <sub>76</sub> N	-0.23
21	42.39	ESI (+)	548.5035 (M+NH <sub>4</sub> ) <sup>+</sup>	548.5035 (M+NH <sub>4</sub> ) <sup>+</sup>	(C <sub>35</sub> H <sub>62</sub> O <sub>3</sub> )+NH <sub>4</sub>	-0.33
22	44.08	ESI (+)	550.6285	550.6285	C <sub>38</sub> H <sub>60</sub> N <sub>1</sub>	-0.2

TABLE 2. Proposed Structures of Identified Compounds (Partial List)

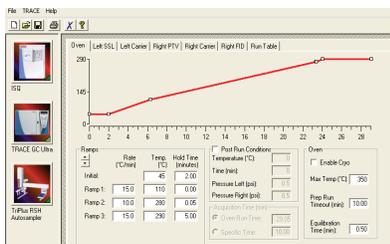


# GCMS Analyses

## Method and Instrumentation

The DI water samples were extracted with Hexane. The samples in 2.0 mL GC vials were introduced in split injection mode into the Thermo Scientific™ TRACE™ Ultra Gas Chromatograph using a Thermo Scientific™ TriPlus RSH™ Autosampler. TG-5ms (30 m x0.25mm x0.25µm) column was used. Compounds were detected and identified with the ISQ single Quad mass spectrometer.

## GCMS Instrument Conditions



GCMS identified lower molecular weight and volatile extractables which complement LCMS results. GCMS results showed that DI water extractions using both techniques were “clean” and more extractables were detected from IPA extractions. Within the four rubber stoppers, sample C&D had more low molecular weight extractables detected by GCMS from both extraction techniques, see Figure 6 and 7.

FIGURE 6. GCMS Chromatogram of IPA\_REFLUX of Sample-D

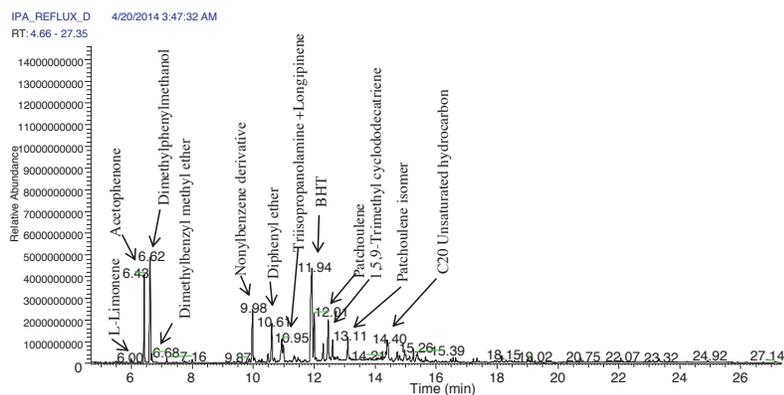
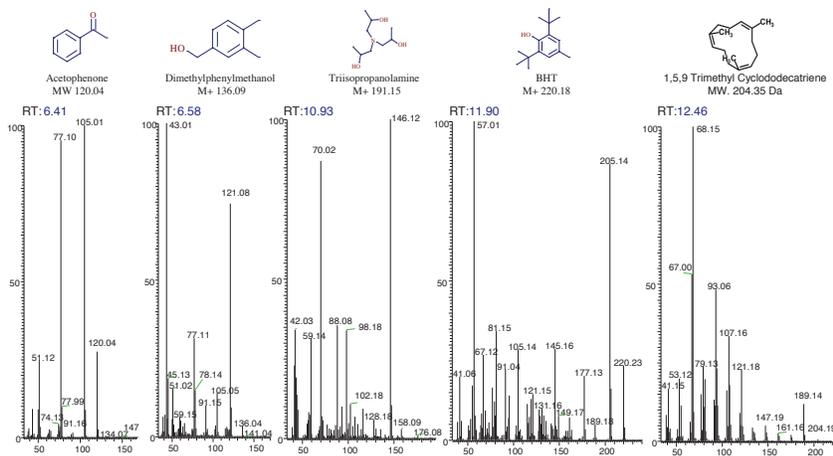


Figure 7. GCMS Spectra of Compounds Identified in IPA\_REFLUX of Sample-D (Partial)



## Summary of Reflux and Speed Extractions using IPA and DI Water

- LCMS and GCMS results show DI water extracts using both Speedy and Reflux techniques were “clean”. “Triisopropanolamine” is the major extractable.
- Complex profiles of IPA extractions were observed from both LCMS and GCMS analysis. Complete extractable list not shown.
- IPA reflux shows higher extraction efficiency compared with IPA speed extraction. However, the speed extractor conditions were not optimized for this study
- Results show that GCMS and LCMS analysis are complementary to each other and necessary to give a fuller picture of the extractable profile.

## ICPMS Analyses

The ICPMS samples were prepared by placing the rubber stoppers 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.

To determine if trace and potentially toxic metals were leached from the stoppers, the USP<232> Class 1 & 2 elements and additional elements which are commonly analyzed by ICP-MS were determined.

The analysis results for the four types of rubber stoppers showed that they are clean of all Class 1 & 2 elements, see Figure 7 for the ICPMS results. In addition, the system control software Qtegra provides a full 21CFR Part 11 tool set to operate under compliant environments.

**FIGURE 7. ICPMS Results for the Four Rubber Stoppers (ppb)**

Element	Sample-1 DI water	Sample-1 Nitric Acid	Sample-2 DI water	Sample-2 Nitric Acid	Sample-3 DI water	Sample-3 Nitric Acid	Sample-4 DI water	Sample-4 Nitric Acid	LOD
75As (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.0233
111Cd (KED)	0.009	0.006	0.003	0.007	0.010	0.009	0.276	0.070	<0.0023
202Hg (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.0054
208Pb (KED)	0.061	0.069	0.018	0.124	0.100	0.106	0.159	0.122	<0.0008
9Be (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.0362
11B (KED)	ND	ND	0.541	ND	0.853	0.596	ND	ND	<0.5229
23Na (KED)	14.326	29.535	8.197	13.575	25.630	20.648	30.074	18.071	<0.1568
24Mg (KED)	1.835	4.091	1.160	1.013	2.802	4.236	2.009	1.531	<0.0231
27Al (KED)	0.420	3.688	0.479	2.325	1.029	3.615	0.867	7.665	<0.32
39K (KED)	8.246	11.185	5.921	5.295	13.580	11.235	16.088	6.645	<1.7964
48Ti (KED)	0.033	0.930	0.033	0.605	0.045	0.202	ND	0.107	<0.0314
51V (KED)	0.526	0.518	ND	ND	ND	ND	0.051	0.044	<0.0339
52Cr (KED)	ND	0.146	ND	0.103	ND	0.241	ND	0.108	<0.0072
55Mn (KED)	0.044	0.161	0.053	0.052	0.045	0.188	0.067	0.102	<0.0065
56Fe (KED)	ND	9.962	ND	3.893	0.570	18.668	0.903	9.600	<0.0175
59Co (KED)	0.001	0.003	0.001	0.001	0.008	0.010	0.011	0.009	<0.0009
60Ni (KED)	0.097	0.123	0.047	0.075	0.254	0.420	0.715	0.281	<0.0063
65Cu (KED)	0.558	0.741	0.237	0.341	0.849	1.576	1.603	1.709	<0.007
66Zn (KED)	13.137	11.712	31.153	43.436	4.049	13.275	81.914	51.445	<0.5173
78Se (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.0781
88Sr (KED)	0.045	0.070	0.037	0.032	0.082	0.087	0.076	0.078	<0.0036
95Mo (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.4285
101Ru (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.0006
103Rh (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.0648
105Pd (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.0038
107Ag (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.0226
121Sb (KED)	0.007	0.017	0.007	0.020	0.017	0.124	0.040	0.052	<0.0012
137Ba (KED)	0.259	0.231	0.165	0.270	1.646	2.420	1.250	1.125	<0.0124
193Ir (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.0003
195Pt (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.0004
205Tl (KED)	ND	ND	ND	ND	ND	ND	ND	ND	<0.0046

## Conclusion

This study demonstrated a comprehensive extractable analysis workflow utilizing multiple techniques: HR-LCMS, GCMS, ICPMS, data processing software, and database searching. This workflow followed recommended analytical methods by PQRI<sup>2</sup>. HS-GCMS was carried out but the data has not been reported.

The DI water and IPA extraction profiles of the four types of medical grade rubber stoppers were quickly established by using this workflow.

The UHPLC/HRAM full MS/HCD MS<sup>2</sup> with rapid polarity switching in a single run data acquisition, coupled with novel database search, significantly increase the confidence and throughput of routine extractable & leachable analysis, in particular for unknown components identification and structure characterization.

The GCMS and LCMS analysis are complementary to each other and necessary to give complete coverage of extractables.

## References

1. FDA CFR 21.94, CFR 66011(b) and 600.11(h), CFR 211.160
2. PQRI "L/E Recommendations to the FDA"  
<http://www.pqri.org/publications/index.asp>

## Acknowledgements

The authors would like to thank Sukhy Toot for her contributions, Qure Medical for providing the rubber stoppers, Smithers Rapra for the extractions, and Buchi for the loan of the speed extractor.

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