Identification of IV Bag Extractables Using GCMS, LC-HRMS, and ICP-MS

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INTRODUCTION

Solutions in IV (Intravenous) bags, such as IV drugs, blood-based products, and parenteral nutrition, are infused directly into the veins of the patients in significant amounts. The quality of IV bag could affect medicine integrity and patient safety. Therefore, extractables and leachables analysis of IV bags and other medicine containers is required by regulatory agencies for product marketing approval.

This poster presents a comprehensive workflow for IV bag extractable analysis using multiple instruments to detect volatile, semivolatile, nonvolatile, and trace elemental impurities.

PURPOSE

To demonstrate a comprehensive workflow for IV bag extractable analysis using Headspace GC-MS, GC-MS, HR-LCMS, and ICP-MS.

MATERIALS AND METHODS

Sample Preparation

Commercially available IV bags were purchased from Amazon. Following PQRI recommendations to the FDA [1] and USP chapter 1663 recommendation [2], the bags were extracted using pH 3 water, pH 9 water, IPA/water (1:1), Ethanol/water (1:1), and phosphate buffered saline (PBS), and placed in a shaker at 50 °C for 7 days. The extract solutions were analyzed using Headspace GC-MS/FID for volatile organic compounds, Liquid-Injection GC-MS/FID for semi-volatile organic compounds, and LC-MS/UV for non-volatile organic compounds.

HR-LCMS ANALYSIS

Liquid Chromatography

The extracts solutions were analyzed directly by HR-LCMS.

LC separations were carried out on the Thermo Ultimate™ 3000 RS UHPLC system consisting of: DGP-3000RS pump, WPS-3000RS sampler, TCC-3000RS column compartment, and DAD-3000RS UV detector.
Column: Thermo Accucore C18, 2.1x100 mm 2.6 µm Column temp: 35 °C.
Mobile phase: A. H_2O/0.05% acetic acid/5 mM ammonium acetate,
B. Acetonitrile/0.05% acetic acid/5 mM ammonium acetate.
Flow rate: 400ul/min with gradient as below.

Time (min)	0	0.5	26.0	36.0	36.1	40.0
Mobil B (%)	5	5	95	95	5	5

Mass Spectrometry

Mass spectrometry analyses were carried out on the Thermo Scientific Q Exactive Plus mass spectrometer using electrospray ionization (ESI). High resolution full scan MS and data-dependent 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.

Ionization mode: positive ESI
Scan Range (Full MS): 120-1200 amu
Ion source: HESI-II
Spray voltage (KV):+3.5
Heated capillary temp (°C): 300
S-lens RF level: 55.0
Heater temp (°C): 430

HR-LCMS RESULTS

High Resolution LCMS Screening with Polarity Switching

The High Resolution Accurate Mass (HRAM) full scan and MS/MS data acquisition with polarity switching was used for extractable analysis. The rapid polarity switching data acquisition ensured the detection of structurally diverse compounds, see Figure 1. It provided complete extractable profile and increased the analysis throughput, see Figure 2. The accurate mass and fine isotope pattern increased the confidence for elemental composition assignments. The information-rich HCD MS/MS spectra assisted the identification and structure elucidation of extractables, especially for unknowns and isobaric component identification, see Figure 3.

FIGURE 1. LCMS Base Peak Chromatogram of IV Bag IPA/H2O Extract

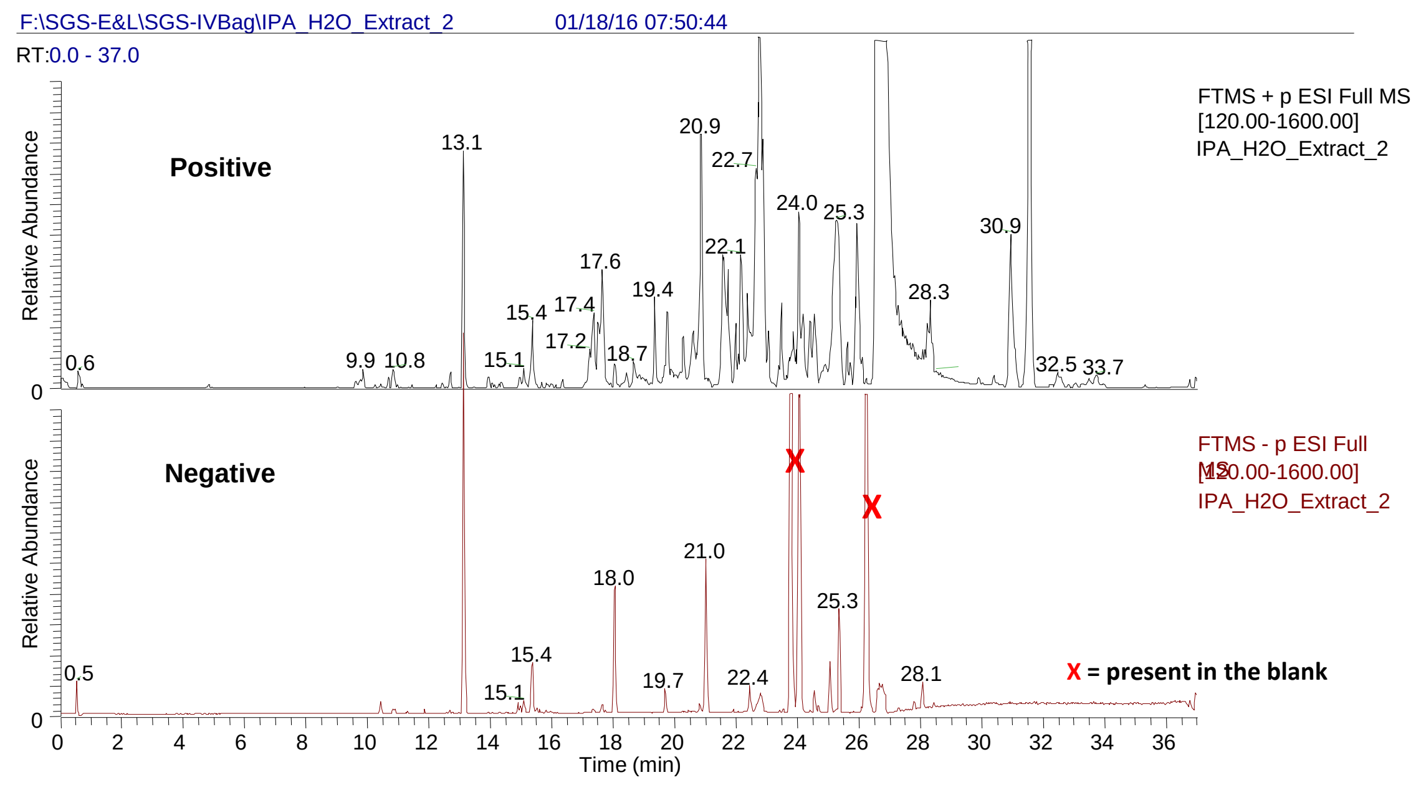


FIGURE 2. HRAM Data with Polarity Switching

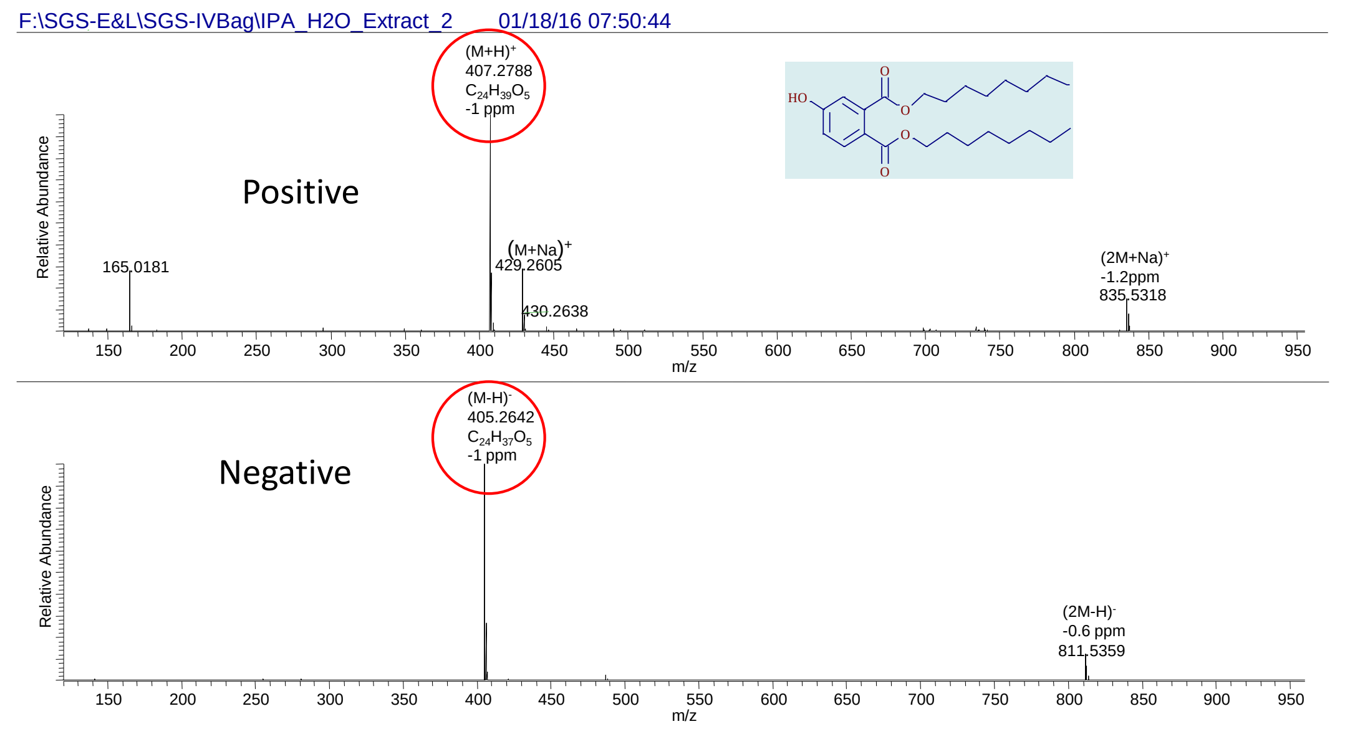
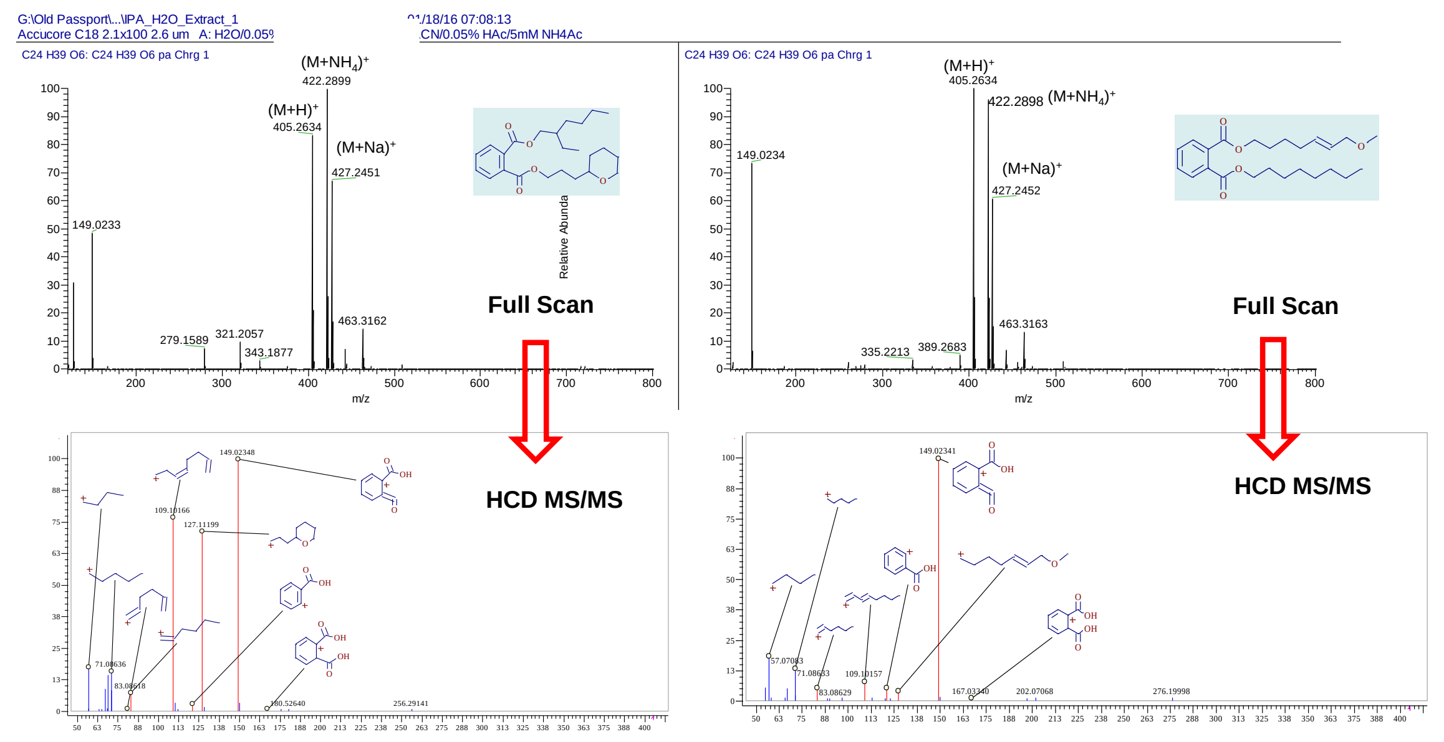


FIGURE 3. Full Scan and HCD MS/MS Data for Component and Structure ID



Data Analysis

Compound Discoverer software and mzCloud spectral library
The HRAM data was processed with Compound Discoverer 2.0 (CD2.0), a small molecule ID software (Thermo Fisher Scientific). The processing workflow was built by following the "New Study and Analysis Wizard", see Figure 4. CD 2.0 detected compounds with "Predicted Formula", followed by automatic online library search against mzCloud and ChemSpider, as well as local E&L compound database. The compounds identified from the mzCloud library can be checked using the mirror plot of ms/ms spectra of identified compound with library standard.

CD 2.0 result view shows the identified components with "Predicted Formula" and corresponding molecular weight. The database search results yield spectra fit and matching score based on mass accuracy and isotope pattern fidelity, see Figure 5. The results were filtered using flexible and versatile "Result Filters" to reduce the false positives.

FIGURE 4. Compound Discoverer 2.0 Node Based Processing Workflow

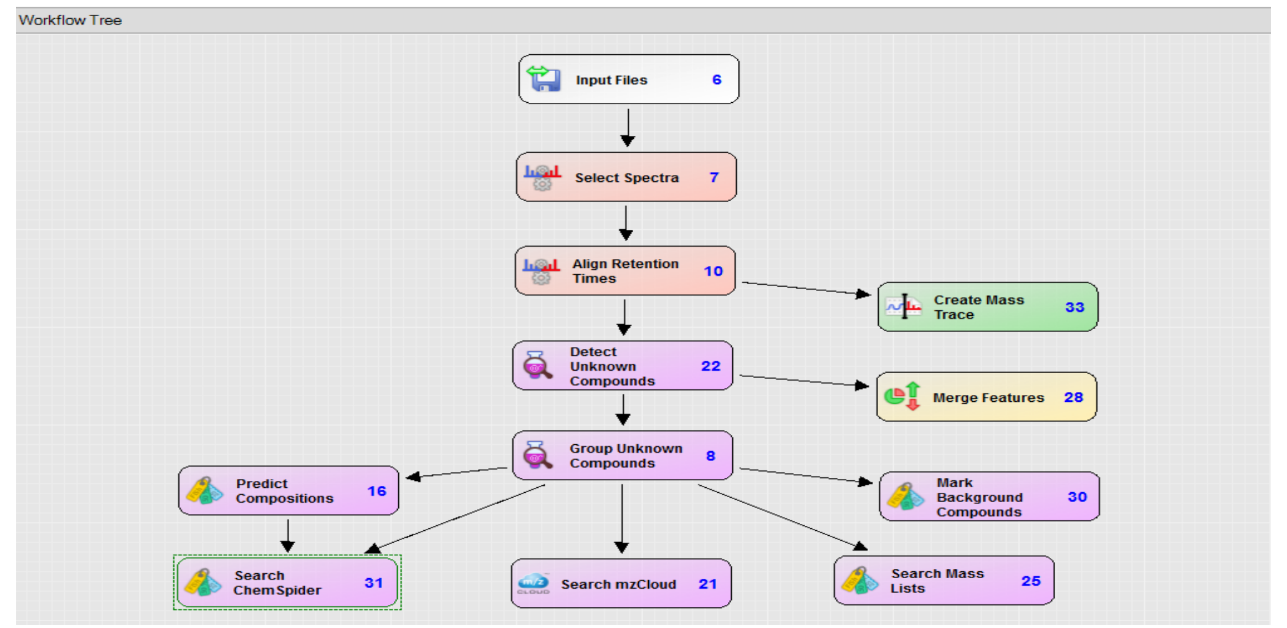
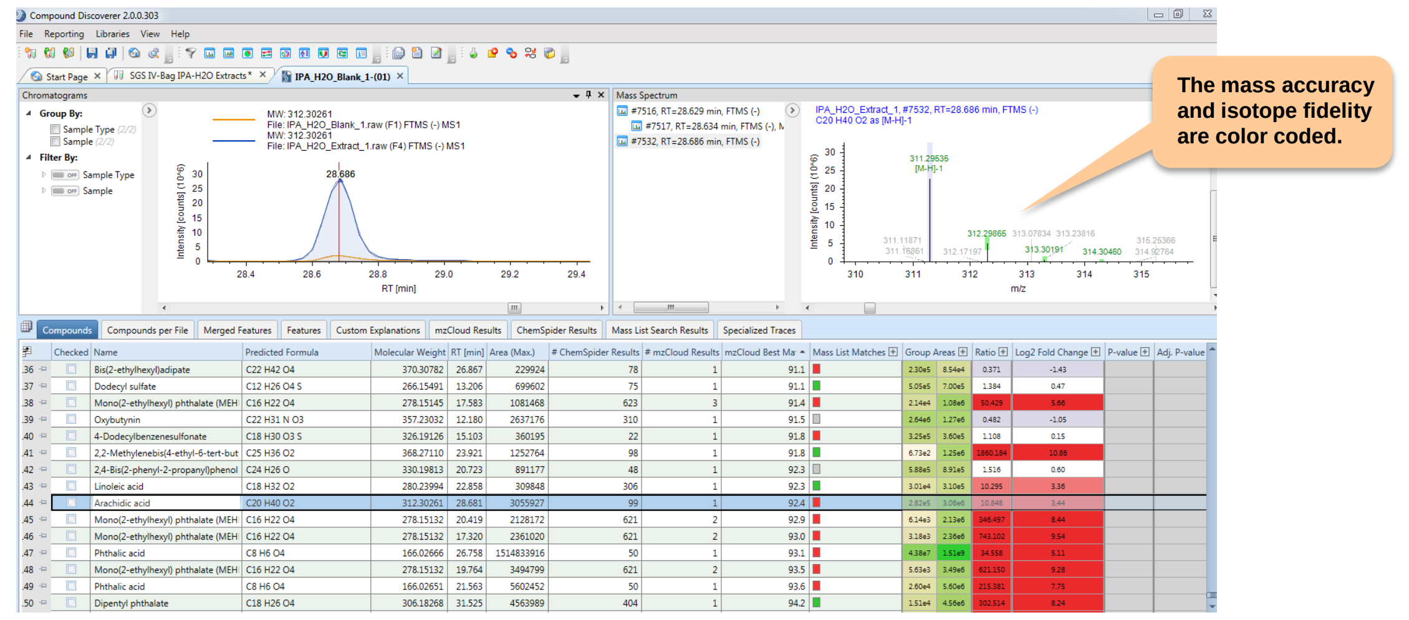


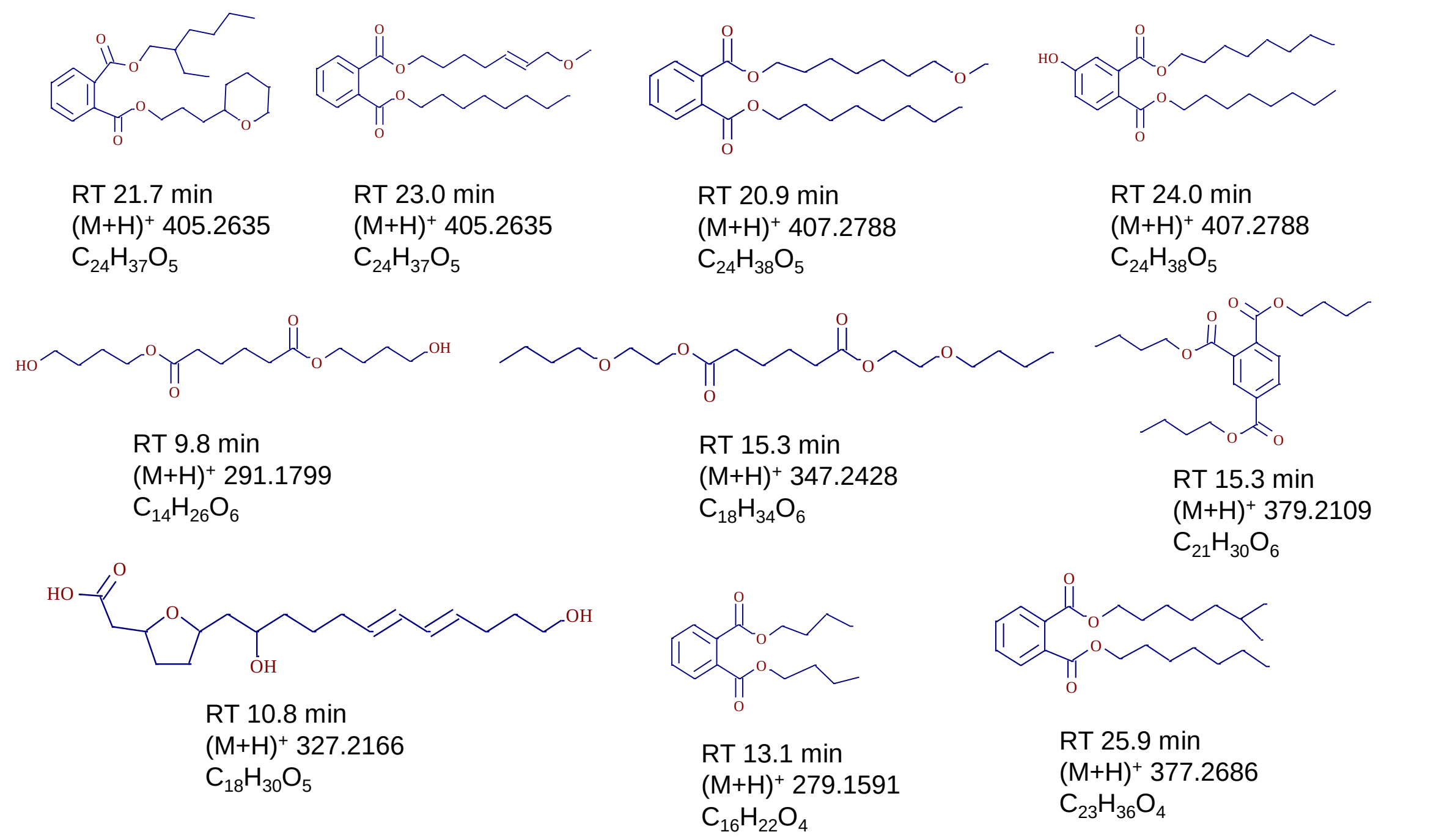
FIGURE 5. Compound Discoverer 2.0 Result View



The mass accuracy and isotope fidelity are color coded.

	Checked	Name	Predicted Formula	Molecular Weight	RT [min]	Area (Max.)	# ChemSpider Results	# mzCloud Results	mzCloud Best Match	Mass List Matches	Group Areas		Ratio	Log2 Fold Change	P-value	Adj. P-value
36	☐	Bis(2-ethylhexyl)adipate	C22 H42 O4	370.30782	26.867	229924	78	1	91.1	🟥	2.30e5	8.54e4	0.371	-1.43		
37	☐	Dodecyl sulfate	C12 H26 O4 S	266.15491	13.206	699602	75	1	91.1	🟥	5.05e5	7.00e5	1.384	0.47		
38	☐	Mono(2-ethylhexyl) phthalate (MEH	C16 H22 O4	278.15145	17.583	1081468	623	3	91.4	🟥	2.14e4	1.08e6	50.429	5.66		
39	☐	Oxybutynin	C22 H31 N O3	357.23032	12.180	2637176	310	1	91.5	⬜	2.64e6	1.27e6	0.482	-1.05		
40	☐	4-Dodecylbenzenesulfonate	C18 H30 O3 S	326.19126	15.103	360195	22	1	91.8	🟥	3.25e5	3.60e5	1.108	0.15		
41	☐	2,2-Methylenebis(4-ethyl-6-tert-but	C25 H36 O2	368.27110	23.921	1252764	98	1	91.8	🟩	6.73e2	1.25e6	1860.184	10.86		
42	☐	2,4-Bis(2-phenyl-2-propanyl)phenol	C24 H26 O	330.19813	20.723	891177	48	1	92.3	⬜	5.88e5	8.91e5	1.516	0.60		
43	☐	Linoleic acid	C18 H32 O2	280.23994	22.858	309848	306	1	92.3	🟩	3.01e4	3.10e5	10.295	3.36		
44	☐	Arachidic acid	C20 H40 O2	312.30261	28.681	3055927	99	1	92.4	🟥	2.82e5	3.06e6	10.848	3.44		
45	☐	Mono(2-ethylhexyl) phthalate (MEH	C16 H22 O4	278.15132	20.419	2128172	621	2	92.9	🟥	6.14e3	2.13e6	346.497	8.44		
46	☐	Mono(2-ethylhexyl) phthalate (MEH	C16 H22 O4	278.15132	17.320	2361020	621	2	93.0	🟥	3.18e3	2.36e6	743.102	9.54		
47	☐	Phthalic acid	C8 H6 O4	166.02666	26.758	1514833916	50	1	93.1	🟥	4.38e7	1.51e9	34.558	5.11		
48	☐	Mono(2-ethylhexyl) phthalate (MEH	C16 H22 O4	278.15132	19.764	3494799	621	2	93.5	🟥	5.63e3	3.49e6	621.150	9.28		
49	☐	Phthalic acid	C8 H6 O4	166.02651	21.563	5602452	50	1	93.6	🟥	2.60e4	5.60e6	215.381	7.75		
50	☐	Dipentyl phthalate	C18 H26 O4	306.18268	31.525	4563989	404	1	94.2	🟩	1.51e4	4.56e6	302.514	8.24		

TABLE 1. Proposed Structures of Extractables in IPA/H2O (Partial List)



RT 21.7 min
$(M+H)^+$ 405.2635
$C_{24}H_{37}O_5$

RT 23.0 min
$(M+H)^+$ 405.2635
$C_{24}H_{37}O_5$

RT 20.9 min
$(M+H)^+$ 407.2788
$C_{24}H_{38}O_5$

RT 24.0 min
$(M+H)^+$ 407.2788
$C_{24}H_{38}O_5$

RT 9.8 min
$(M+H)^+$ 291.1799
$C_{14}H_{26}O_6$

RT 15.3 min
$(M+H)^+$ 347.2428
$C_{18}H_{34}O_6$

RT 15.3 min
$(M+H)^+$ 379.2109
$C_{21}H_{30}O_6$

RT 10.8 min
$(M+H)^+$ 327.2166
$C_{18}H_{30}O_5$

RT 13.1 min
$(M+H)^+$ 279.1591
$C_{16}H_{22}O_4$

RT 25.9 min
$(M+H)^+$ 377.2686
$C_{23}H_{36}O_4$

Unknown Compound Identification Using "Custom Explanation" and "FISh Scoring" (FISh stands for Fragment Ion Search)

The identified unknown compounds were added to the "Custom Explanations" table. Based on the predicted formula, molecular weight, and ms/ms fragmentation, putative structures were proposed in "Custom Explanation Editor", then the "FISh Scoring" feature was used to search the embedded "Fragments and Mechanisms" library. Any matching fragments were automatically annotated with fragment structures, and the "FISh Coverage" score indicated the percentage of fragment ion matching between experimental data and fragmentation libraries.

GC-MS ANALYSIS

Headspace GC-MS Results:

Headspace GC-MS analysis was conducted on Agilent G1888 Headspace sampler , Agilent 6890N GC, and Agilent 5973 MS to identify volatile organic.

Cyclohexanone was found in all extracts, which was also shown by liquid injection GC-MS analysis. Unexpectedly, IPA was found in all aqueous extracts. Further investigation showed that the IPA found in aqueous extracts was volatile small molecule compounds migrate/penetrate through plastic bags from IPA/Water extraction solvent, which was present in the same oven during incubation.

The overlap of extractable profile by Headspace-GC-MS and Liquid Injection GC-MS has shown the completeness of the volatile and semi-volatile organic compound profile in the methods.

Liquid-injection GC-MS

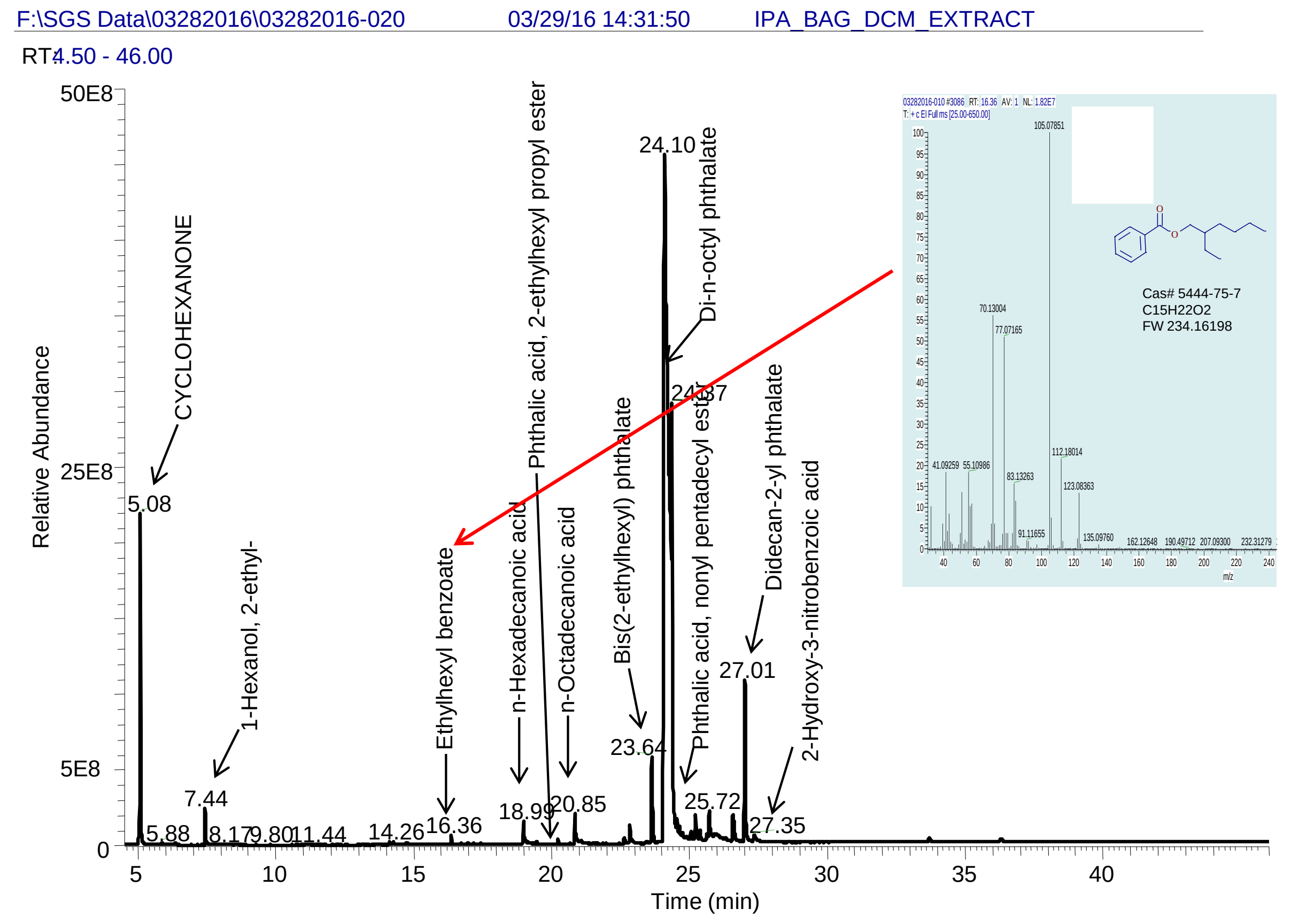
Liquid-Injection GC-MS analysis was conducted to identify semivolatile organic compounds using Thermo Trace 1310 GC and ISQ single Quad MS. NIST library was used to assist identification. The IPA/Water extract has shown the highest concentration of extractable compounds, especially hydrophobic compounds, compared to other extracts (pH 3, pH 9 PBS, and Ethanol/Water). The major peak observed from all extracts is Bis (2-ethylhexyl) phthalate (DEHP), which is the primary plasticizer of this PVC bag. Most of the extractable compounds are phthalates, degradants from DEHP, and lubricants, see Figure 6.

GC-MS identified the volatile and semivolatile compounds. The result was complementary to the LCMS result.

Liquid-Injection GC-MS Conditions

Gas Chromatograph: Thermo Trace 1310 GC
Mass Spectrometer: Thermo Single Quad ISQ
Column: TG-5HT (30 m × 0.25 mm × 0.10 µm)
Temperature Program: not show
Total Run Time: 45 min
Carrier Gas: Helium
Carrier Gas Flow: 1.5 mL/min
Injector: splitless (for 1.0 min)
Injection Volume: 1.0 µL

FIGURE 6. GC-MS Chromatogram of IPA/Water Extract (with DCM Extraction)



ICP-MS RESULTS

The ICPMS samples were prepared by filling the IV bags with 250 ml of 1% nitric acid and 250 ml DI water, respectively. The filled bags were kept 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 iCAP Q was set up using Helium gas in the collision cell in KED mode.

The USP<232> Class1 & 2 elements and additional elements (total 66) were determined in this analysis. The results demonstrated that the IV bags were clean, containing no toxic elements, see Table 2. The system control software Qtegra provides full 21CFR Part 11 toolset to operate under compliant environments. It is compatible with USP 233 & 232 requirements.

Supported 21 CFR 11 Sections	
1.10	Controls for closed systems
11.30	Controls for open systems
11.50	Signatures manifestations
11.70	Signature / record linking
11.100	General requirements for electronic signatures
11.200	Electronic signatures components and controls
11.300	Controls for identification codes / passwords

TABLE 2. ICP-MS Result (ppb)

Element	IV DI	IV Acid	Element	IV DI	IV Acid	Element	IV DI	IV Acid
66Zn	65.32	81.14	60Ni	ND	ND	157Gd	ND	ND
44Ca	20.49	23.90	73Ge	ND	ND	159Tb	ND	ND
24Mg	1.48	3.38	78Se	ND	ND	163Dy	ND	ND
23Na	1.06	0.74	85Rb	ND	ND	165Ho	ND	ND
48Ti	0.04	0.06	89Y	ND	ND	166Er	ND	ND
88Sr	0.02	0.03	90Zr	ND	ND	169Tm	ND	ND
137Ba	0.02	0.01	93Nb	ND	ND	172Yb	ND	ND
63Cu	0.01	0.03	95Mo	ND	ND	175Lu	ND	ND
55Mn	ND	0.01	101Ru	ND	ND	178Hf	ND	ND
75As	ND	ND	103Rh	ND	ND	181Ta	ND	ND
111Cd	ND	ND	105Pd	ND	ND	182W	ND	ND
202Hg	ND	ND	107Ag	ND	ND	185Re	ND	ND
208Pb	ND	ND	115In	ND	ND	189Os	ND	ND
7Li	ND	ND	118Sn	ND	ND	193Ir	ND	ND
9Be	ND	ND	121Sb	ND	ND	195Pt	ND	ND
11B	ND	ND	125Te	ND	ND	197Au	ND	ND
27Al	ND	ND	133Cs	ND	ND	205Tl	ND	ND
39K	ND	ND	139La	ND	ND	209Bi	ND	ND
45Sc	ND	ND	140Ce	ND	ND	232Th	ND	ND
51V	ND	ND	141Pr	ND	ND	238U	ND	ND
52Cr	ND	ND	146Nd	ND	ND			
56Fe	ND	ND	147Sm	ND	ND			
59Co	ND	ND	153Eu	ND	ND			

CONCLUSIONS

This study demonstrated a comprehensive extractable analysis workflow utilizing multiple techniques: HR-LCMS, GCMS, ICPMS, and data processing software with database searching.

- The high resolution MS screening method using full MS and HCD MS2 with rapid polarity switching in a single run increased the confidence and throughput of routine extractable & leachable analysis.
- Data processing software and spectral library facilitate compound structure ID and characterization.
- Results show that GCMS and LCMS analysis are complementary to each other and necessary to give a fuller picture of the extractable profile.
- *iCAP Q ICP-MS control software Q tegra provides full 21CFR Part 11 toolset to operate under compliant environments, and is compatible with USP 233 & 232 requirements.

REFERENCES

1. PQRI "L/E Recommendations to the FDA" http://www.pqri.org/publications/index.asp
2. USP Chapter 1663 "Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems" August 2015