Food Contact Material (FCM) Migration Study using HR-LCMS and Novel Software Database Suite

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Introduction

To ensure consumer safety, food/beverage packaging companies are required to conduct Food Contact Material (FCM) migration studies following regulatory guidelines [1]. FCM migration study is the key component of marketing approval document "Food Contact Notification (FCN)" by authorities around the world. FCM migration study requires components identification, characterization, and quantitation for safety assessment. Because of sample complexity and the unknown, unexpected nature of some components, advanced analytical instruments, combined with good software and databases, can significantly alleviate the challenge of FCM migration study.

This study presents a migration study workflow for food packaging bags, both non-gamma-irradiated and gamma-irradiated, using HR-LCMS and database search.

LCMS Analysis

Sample Preparation

Beverage bags non-irradiated and gamma irradiated at 10 kGy were extracted using 3% acetic acid and 50% ethanol food simulants for 12 days at 40°C [2].

Liquid Chromatography

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 um. Column Temp: 35ºC.

Injection Volume: 10 ul

LC Mobile phase: A: H_2O/0.1FA B: ACN/0.1% FA , flow rate: 400ul/min.

Gradient: Time (min.)	0	2	28	39	39.1
Mobile B (%)	5	15	90	90	5

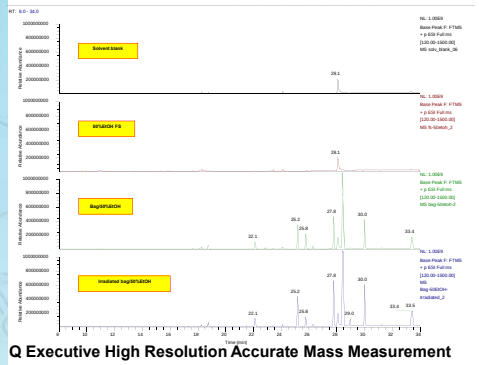
Mass Spectrometry

MS analyses were carried out on Thermo Scientific™ Q Exactive™ Plus mass spectrometer using electrospray ionization (ESI). High resolution accurate mass (HRAM) full scan MS and top 3 data-dependent HCD MS/MS data were collected at resolving power of 70,000 and 17,500 (FWHM m/z200) with polarity switching. The scan range is 150-1500 amu. Stepped HCD normalized collision energy: 20, 40, and 60. Quantitation was conducted using SIM scan at isolation window 5 amu and resolution 70,000.

Results

The HRAM full scan data allow confident component identification and elemental composition assignment. The infomraiton-rich HCD MS/MS fragments provide valuable data for structure elucidation. Rapid positive/negative polarity switching gives additional information and confidence in component detection and characterization.

FIGURE 2. Base Peak Chromatograms of 50% EtOH Extraction Samples ESI (+)



Q Executive High Resolution Accurate Mass Measurement

Q Exactive High Resolution Accurate Mass (HRAM) data provides ultimate confidence for qualitative and quantitative analysis. The very sensitive, rapid polarity switching ensure detection of structurally diverse compounds at all levels.

SIEVE Software for Component Extraction and Differential Analysis

SIEVE, a differential analysis software, was used for component extraction. Figure 4 shows the base peaks alignment of extracted components. The extracted components are then searched against "Chemspider" or Thermo E&L Compound Database to identify each component. A hit list is generated. A good database can improve hit rate and quickly identify the known compounds through HRAM data and ms/ms fragment ions matching.

Figure 1. HR-LCMS Analysis Workflow for FCM Migration Study

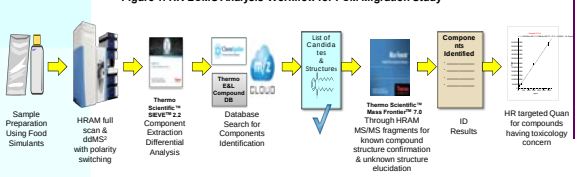


FIGURE 3. High Mass Accuracy for Positive/Negative Mode with Polarity Switching

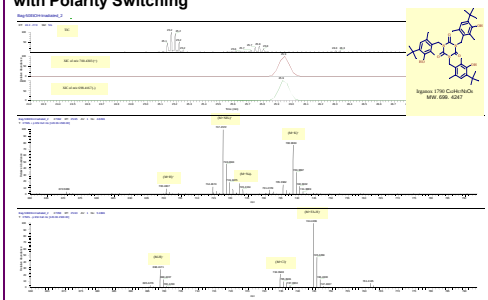
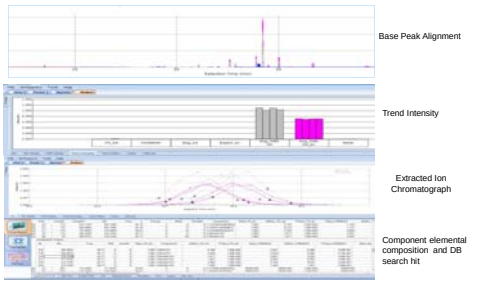


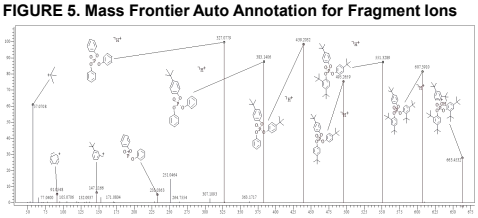
FIGURE 4. SIEVE software for component extraction and Differential Analysis



Mass Frontier and MzCloud for Structure Elucidation

For unknown components, multiple possible structures were obtained for each component through ChemSpider database or other database searching, in order to determine the correct structures. "Mass Frontier™",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. A high degree of correlation between predicted and experimental fragments confirmed the proposed structure. Mass Frontier then automatically annotated the matching fragments based on library search results, see Figure 5. Mass Frontier can build customized libraries.

FIGURE 5. Mass Frontier Auto Annotation for Fragment Ions



mzCloud Spectral Database Searching

A search was also conducted with "mzCloud", a high resolution spectral database. 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 6 shows identification of Erucamide using the ms/ms spectrum search feature.

FIGURE 6. mzCloud Spectral Database Searching for Erucamide

FIGURE 7. Base Peak Chromatogram of 50% EtOH extraction for γ-Irradiated Bag

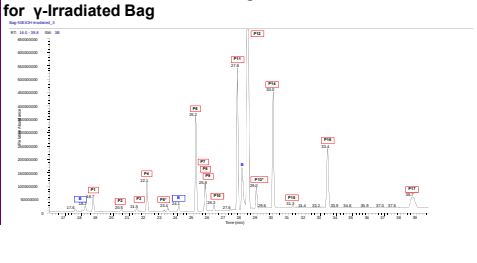
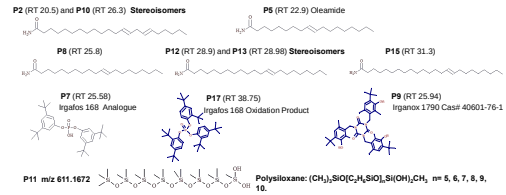


TABLE 1. Major Components Identified from 50% EtOH Extraction of γ-Irradiated Bag

Peak ID	RT (min.)	Measured	Calculated	Elemental Composition	Error (ppm)
P1	18.76	371.1006 (-H$_2$O)	371.1012	$C_{18}H_{35}O_5Si_2$	-1.3
		389.1112 (+H)	389.1118	$C_{18}H_{37}O_6Si_2$	-1.5
		411.0931(+Na)	411.0937	$C_{18}H_{36}O_6Si_2Na$	-1.2
P2	20.50	336.3255 (+H)	336.3261	$C_{21}H_{42}ON$	-1.7
P3	21.49	352.3206 (+H)	352.3210	$C_{21}H_{42}O_2N$	-1.2
P4	22.13	445.1196 (-H$_2$O)	445.1200	$C_{24}H_{45}O_5Si_2$	-0.3
		463.1303 (+H)	463.1306	$C_{24}H_{47}O_6Si_2$	-0.5
		485.1125 (+Na)	485.1125	$C_{24}H_{46}O_6Si_2Na$	-0.1
P5*	22.90	282.2787 (+H)	282.2791	$C_{18}H_{35}ON$	-1.5
P6	25.19	519.1386 (-H$_2$O)	519.1388	$C_{24}H_{43}O_7Si_2$	-0.4
		537.1490 (+H)	537.1494	$C_{24}H_{45}O_8Si_2$	-1.0
		554.1758 (+NH$_4$)	554.1759	$C_{24}H_{48}O_8NSi_2$	-0.7
P7	25.58	473.2823 (-H)	473.2826	$C_{28}H_{41}O_4P$	-0.6
P8	25.80	310.3099 (+H)	310.3104	$C_{21}H_{41}ON$	-1.7
P9	25.94	700.4307(+H)	700.4320	$C_{45}H_{57}NiO_6$	-1.8
P10	26.30	336.3254 (+H)	336.3261	$C_{23}H_{43}ON$	-2.0
P11	27.78	593.1567 (-H$_2$O)	593.1576	$C_{28}H_{49}O_8Si_3$	-1.5
		611.1672 (+H)	611.1682	$C_{28}H_{51}O_9Si_3$	-1.5
		628.1941(+NH$_4$)	628.1947	$C_{28}H_{54}O_9NSi_3$	-1.0
P12	28.43	338.3413 (+H)	338.3417	$C_{23}H_{45}ON$	-1.1
P13	28.98	338.3409 (+H)	338.3417	$C_{23}H_{45}ON$	-2.6
P14	30.01	667.1752 (-H$_2$O)	667.1764	$C_{34}H_{51}O_8Si_3$	-2.0
		685.1835 (+H)	Very weak		
		702.2124 (+NH$_4$)	702.2135	$C_{34}H_{56}O_8NSi_3$	-1.6
P15	31.27	366.3725 (+H)	366.3730	$C_{25}H_{47}ON$	-1.5
P16	33.46	741.1945 (-H$_2$O)	741.1952	$C_{35}H_{53}O_8Si_4$	-1.8
		776.2311(+NH$_4$)	776.2323	$C_{35}H_{58}O_8NSi_4$	-1.5
P17	38.75	663.4530 (+H)	663.4537	$C_{45}H_{59}O_4P$	-1.3

FIGURE 8. Proposed Structures of Identified Compounds (Partial List)



High Resolution Quantitation of Antioxidant Irganox 1035

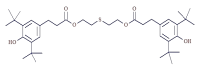
Quantitation is required for certain FCM. In this study, Antioxidant Irganox1035 in food simulant 50% EtOH was used to demonstrate the Quan capability of Q Exactive Plus.

3,5-Bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoic acid thiodi-2,1-ethanediyl ester

CAS# 41484-35-9

Molecular formula: C38H58O6S

Formula weight: 642.3954



Stock solution: 1mg/mL in IPA. Working solutions were prepared by serial dilution of stock using food simulant 50%EtOH, see calibration curve for concentrations. The calibration curve was generated over the range of 0.05-100ppb, linear regression and 1/x weighting. The results show an excellent LOD 90.05ppb) and linearity, see Figure 9 and Table 2 Negative mode data not shown

FIGURE 9. LCMS Chromatogram of IPA Reflux of Sample A

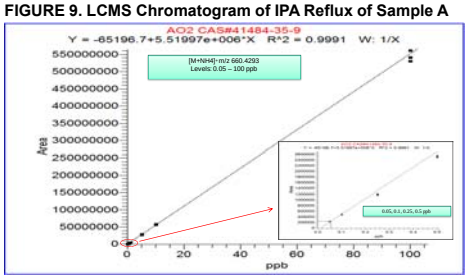


TABLE 2. Calibration Table for Antioxidant Irganox 1035

	FileName	Level	RT	Response	Specified Co	Calculated C	% Diff	% RSD	% CV	Integration Type	Exclude
1	AO2_EtOH_5	1	4.12	240350	0.050	0.055	10.71	0.87	1.10	Method Settings	
2	AO2_EtOH_5	1	4.12	238715	0.050	0.055	10.11	0.87	1.10	Method Settings	
3	AO2_EtOH_5	1	4.12	234266	0.050	0.054	8.15	0.87	1.10	Method Settings	
4	AO2_EtOH_5	1	4.13	236663	0.050	0.055	9.37	0.87	1.10	Method Settings	
5	AO2_EtOH_1	2	4.12	496078	0.100	0.101	1.00	1.84	2.09	Method Settings	
6	AO2_EtOH_1	2	4.12	491533	0.100	0.101	0.92	1.84	2.09	Method Settings	
7	AO2_EtOH_1	2	4.13	476076	0.100	0.098	-1.54	1.84	2.09	Method Settings	
8	AO2_EtOH_1	2	4.13	476168	0.100	0.098	-1.92	1.84	2.09	Method Settings	
9	AO2_EtOH_2	3	4.12	1169472	0.250	0.224	-10.35	1.80	1.90	Method Settings	
10	AO2_EtOH_2	3	4.13	1176348	0.250	0.225	-10.03	1.80	1.90	Method Settings	
11	AO2_EtOH_2	3	4.13	1157576	0.250	0.229	-8.45	1.80	1.90	Method Settings	
12	AO2_EtOH_2	3	4.13	1219431	0.250	0.233	-6.91	1.80	1.90	Method Settings	
13	AO2_EtOH_5	4	4.12	2367964	0.500	0.476	-4.74	1.18	1.21	Method Settings	
14	AO2_EtOH_5	4	4.13	2491326	0.500	0.463	-7.37	1.18	1.21	Method Settings	
15	AO2_EtOH_5	4	4.13	2520764	0.500	0.468	-6.31	1.18	1.21	Method Settings	
16	AO2_EtOH_5	4	4.13	2512962	0.500	0.467	-6.60	1.18	1.21	Method Settings	
17	AO2_EtOH_1	5	4.12	5309112	1.000	0.969	-3.15	0.69	0.70	Method Settings	
18	AO2_EtOH_1	5	4.14	5330980	1.000	0.978	-2.15	0.69	0.70	Method Settings	
19	AO2_EtOH_1	5	4.12	5299019	1.000	0.972	-2.84	0.69	0.70	Method Settings	
20	AO2_EtOH_1	5	4.13	5346435	1.000	0.980	-1.96	0.69	0.70	Method Settings	
21	AO2_EtOH_5	6	4.12	28669197	5.000	5.206	4.12	0.77	0.77	Method Settings	
22	AO2_EtOH_5	6	4.12	28433658	5.000	5.167	3.36	0.77	0.77	Method Settings	
23	AO2_EtOH_5	6	4.13	28579155	5.000	5.189	3.76	0.77	0.77	Method Settings	
24	AO2_EtOH_5	6	4.12	28169533	5.000	5.115	2.30	0.77	0.77	Method Settings	
25	AO2_EtOH_1	7	4.12	58853504	10.000	10.674	6.74	1.62	1.62	Method Settings	
26	AO2_EtOH_1	7	4.14	58453367	10.000	10.601	6.01	1.62	1.62	Method Settings	
27	AO2_EtOH_1	7	4.13	58074955	10.000	10.532	5.32	1.62	1.62	Method Settings	
28	AO2_EtOH_1	7	4.12	56689618	10.000	10.282	2.82	1.62	1.62	Method Settings	
29	AO2_EtOH_1	8	4.13	541106496	100.000	98.039	-1.96	2.79	2.79	Method Settings	
30	AO2_EtOH_1	8	4.14	530488121	100.000	96.115	-3.89	2.79	2.79	Method Settings	
31	AO2_EtOH_1	8	4.11	560964681	100.000	101.676	1.68	2.79	2.79	Method Settings	
32	AO2_EtOH_1	8	4.13	561457863	100.000	101.726	1.73	2.79	2.79	Method Settings	

Conclusion

This study demonstrated an extractable analysis workflow for food contact material migration study for identification and quantitation. The UHPLC/HRAM full MS/HCD MS^2 with polarity switching on the fly data acquisition, coupled with novel database search, significantly increase the confidence and throughput of routine extractable analysis, in particular for unknown components identification and structure characterization.

References

1. FDA CFR 21.94, CFR 66011(b) and 600.11(h), CFR 211.160
2. FDA: Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations April 2002; December 2007