Characterizing Extractables from Common Pharmaceutical Packaging Material by High Resolution Time-of-Flight Mass Spectrometry and Enhanced Gas Chromatography Separations

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LECO EMPOWERING RESULTS

INTRODUCTION

The characterization of extractable and leachable components from a wide range of materials is an important area of research. Information about extractable and leachable components from packaging and delivery devices for pharmaceutical products is a particular area of growing interest. Analytical testing results on this topic are part of many regulatory submission requirements to the FDA. USP 1663 provides guidance on extractables testing and a variety of analytical approaches can meet compliance. High resolution MS is often considered necessary for identification of unknowns, and sample complexity and low-level detection continue to challenge these analyses. Here, we demonstrate a workflow that uses comprehensive two-dimensional gas chromatography (GCxGC) with HR-TOFMS to help address these challenges.

METHOD

Representative extract samples were prepared from various materials that are commonly used in packages and closures for pharmaceutical products. Butyl rubber stoppers and plastic syringes (with and without rubber components) were extracted with methylene chloride at room temperature for 72 h. The extracts were analyzed by GCxGC-HR-TOFMS (Pegasus® HRT⁺ 4D, LECO), as described in Table 1. An alkane standard was also analyzed for retention index (RI) determinations. EI and CI (methane) data were collected with a multi-mode ionization source and used to support identifications.

Table 1. Instrument Conditions

AS	**LECO L-PAL3 Autosampler**
Injection	1 µL
GCxGC	**LECO GCxGC QuadJet™ Thermal Modulator**
Inlet	280 °C, splitless
Carrier Gas	He @ 1.40 mL/min, constant flow
Columns	Column 1: Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek) Column 2: Rxi-17Sil MS, 0.9 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Temperature Program	2 min 50 °C, ramp 8 °C/min to 340 °C, hold 5 min Secondary Oven: + 20 °C
Modulation	3 s with temperature maintained +15 °C relative to 2nd oven
Transfer Line	350 °C
MS	**LECO Pegasus HRT⁺ 4D**
Source	LECO MMS™ (EI and CI, with methane)
Ion Source Temp	250 °C (EI) and 165 °C (CI)
Mass range	35-900 m/z (EI) and 60-900 (CI)
Acquisition Rate	125 spectra/s

GCxGC-TOFMS
LECO *Pegasus* HRT⁺ 4D

ENHANCED SEPARATION WITH GCxGC

GCxGC improves the peak capacity of a separation, which allows for chromatographically isolating more analytes from each other and from interferences. This can help uncover new analyte peaks, provide spectra with fewer interferences, and lead to more identified analytes.

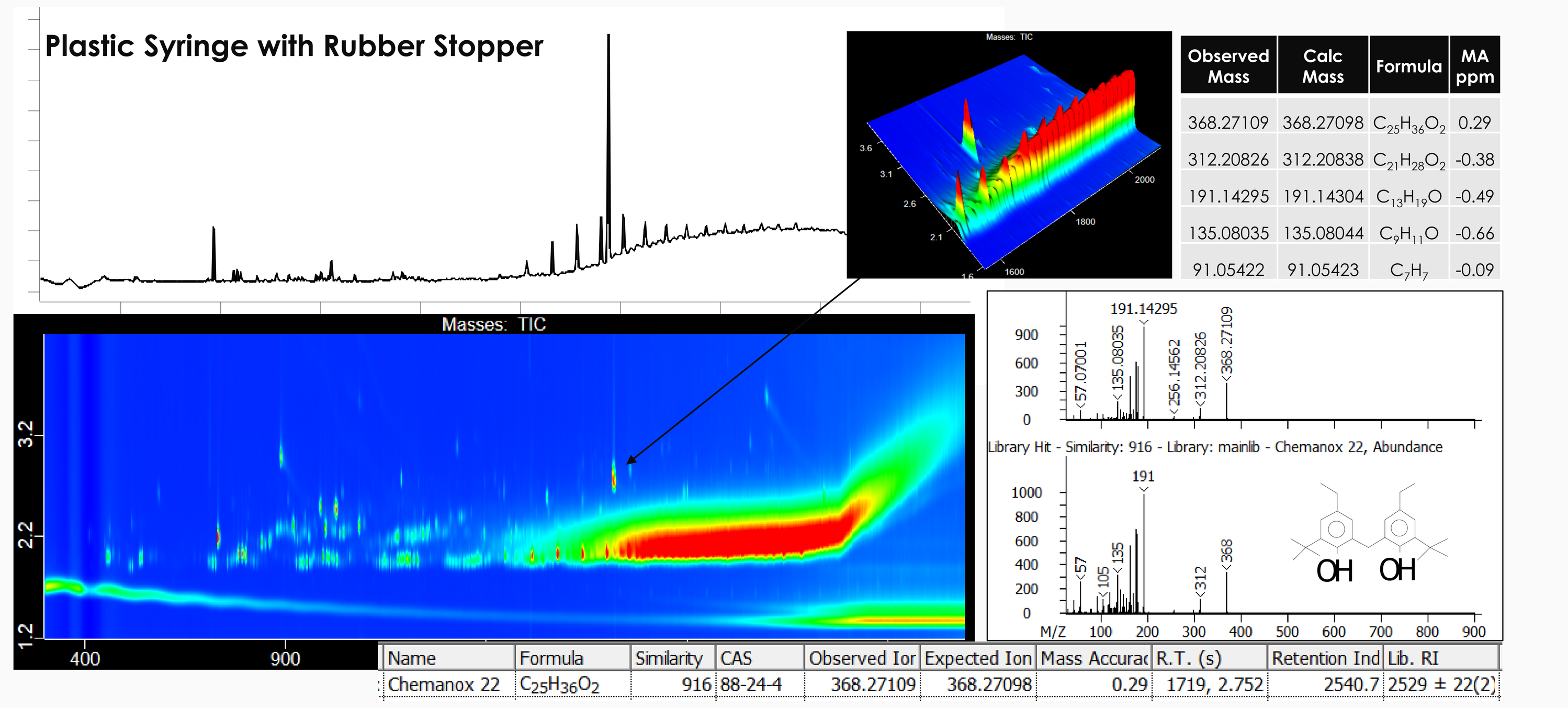

Figure 1. GC and GCxGC chromatograms for an extract of a plastic syringe indicate improved chromatographic separation. More analytes are isolated from each other in chromatographic space and separated from interferences. Additionally, structured chromatograms and elution order in the second dimension add support to identifications along with similarity score, RI in the first dimension, and accurate mass molecular formula and fragment determinations. As an example, Chemanox 22, a rubber antioxidant, can be observed separated from background interferences.

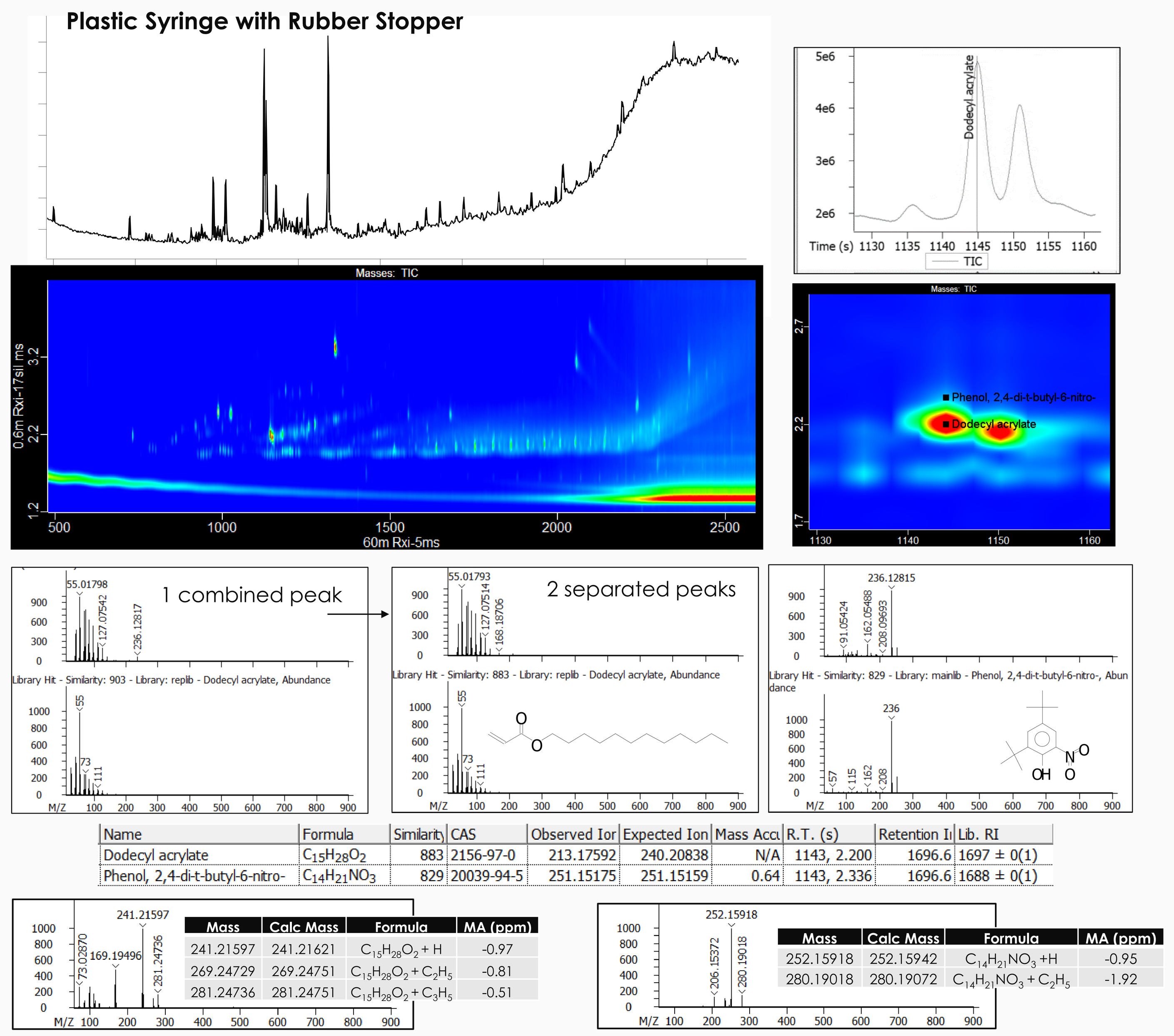

Figure 2. In some instances, GCxGC helps uncover new analytes that were hidden in a GC separation. Here, a phenol compound and dodecyl acrylate completely coelute and are combined as one peak in the 1D separation. With GCxGC, the analytes are chromatographically separated in the second dimension, and both were determined. This revealed more information than could be determined with just GC. Additionally, CI data added molecular ion support for dodecyl acrylate that did not have a molecular ion in the EI data and also confirmed molecular ion for 2,4-di-t-butyl-6-nitrophenol.

IMPROVED IDENTIFICATIONS

Incorporating EI and CI accurate m/z information from HR-MS with GCxGC, which generally provides cleaner spectra for interpretation, can yield better identifications.

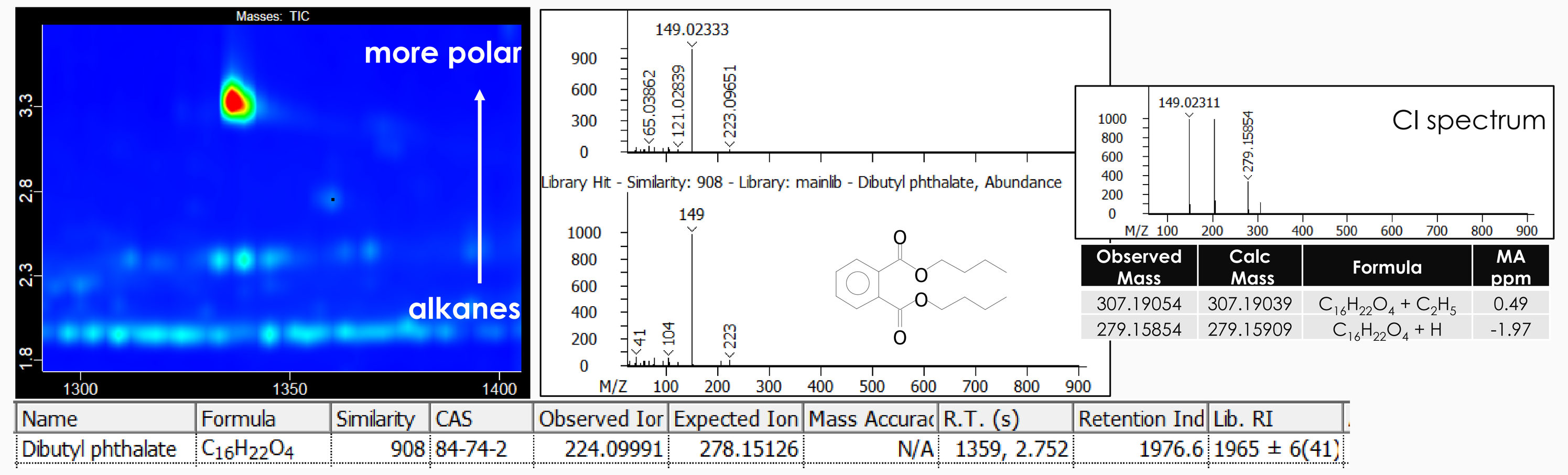

Figure 3. GCxGC provides improved chromatographic separations, cleaner spectra, and structured chromatograms, as shown here. HR-MS, both EI and CI, add crucial identification information. In instances where a molecular ion is not present in the EI data, the CI data can often support the formula determination, as shown here for a phthalate, observed in a plastic syringe.

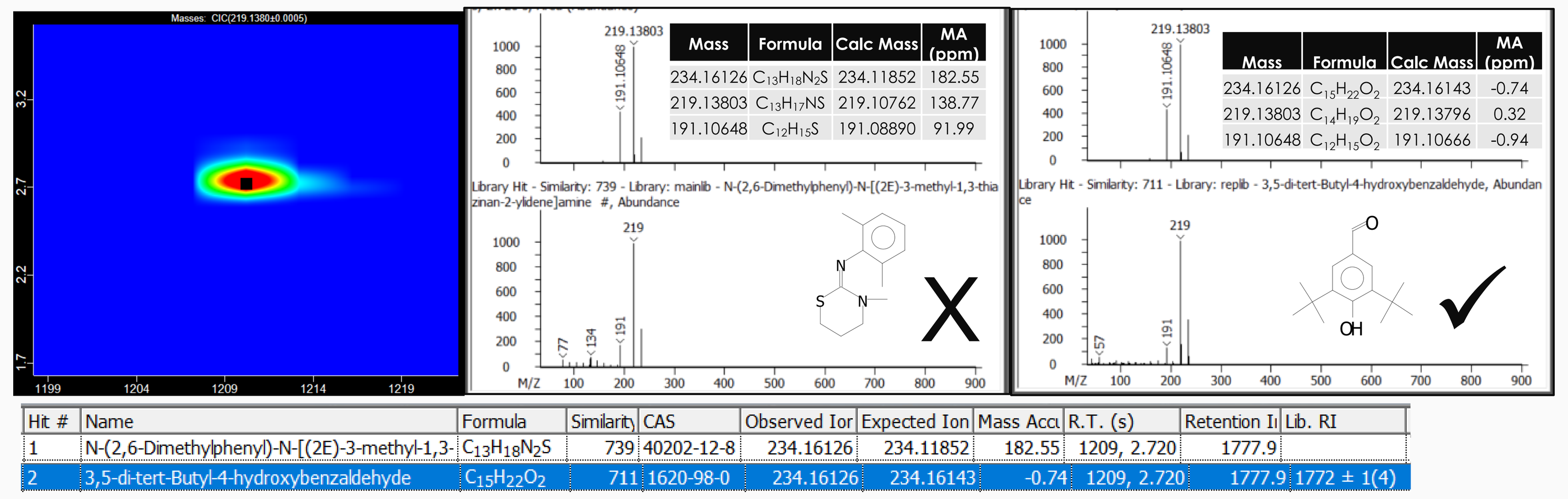

Figure 4. Accurate mass and formulae determinations can also allow for selecting an improved spectral match from the library database. In this case from a plastic syringe, both library matches are supported by nominal mass fragmentation patterns, but the formula for the first library hit was not well-supported and the second library hit was.

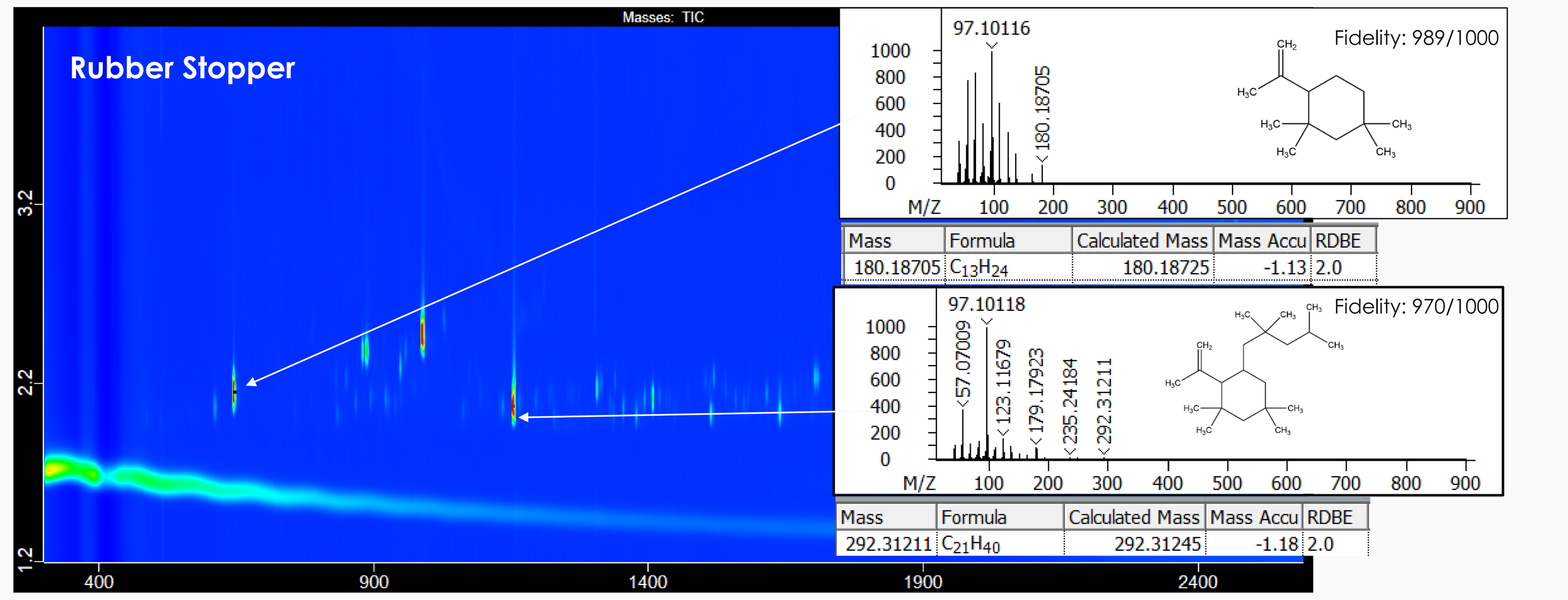

Figure 5. Accurate mass information can also help determine formulae for features that are not present in library databases. In this example, butyl oligomers that were not in NIST library databases were observed in a rubber stopper extract. Accurate mass information was used to determine the formula and elution in the structured space supported the identifications.

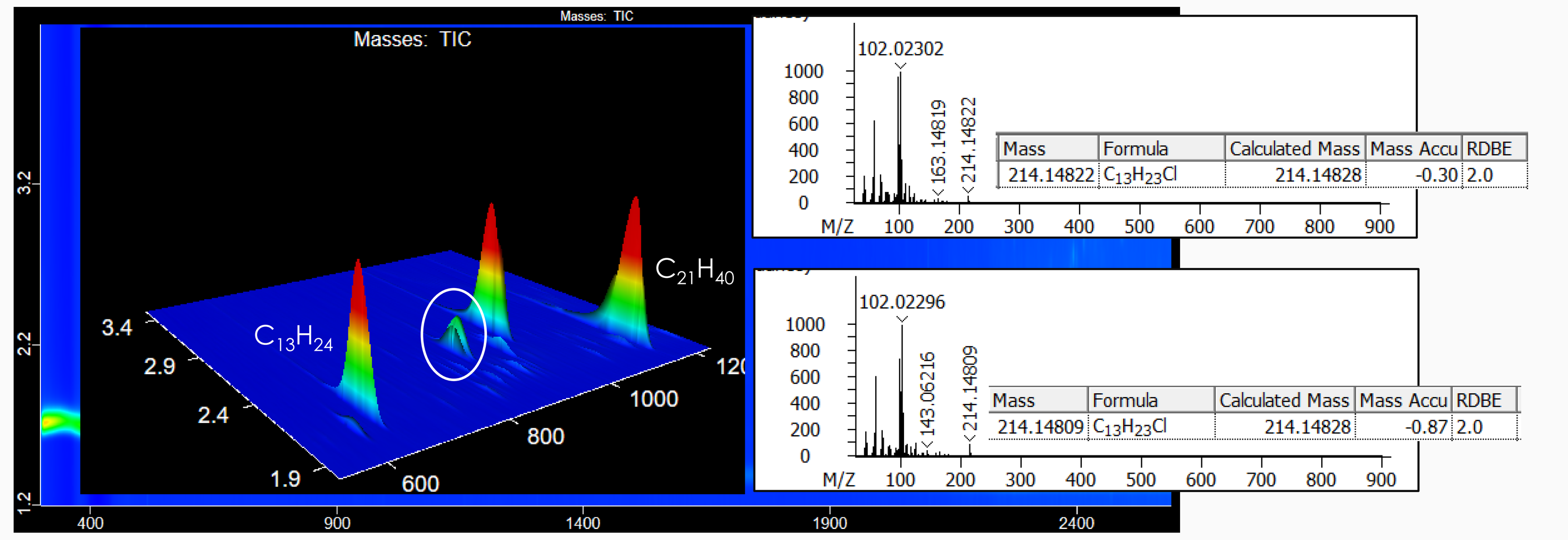

Figure 6. Chlorinated butyl oligomers that were not in NIST library databases were also observed in a rubber stopper extract. Accurate mass information was used to determine the formula and elution in the structured space supported the identifications.

SAMPLE CHARACTERIZATION

A variety of samples were evaluated with GCxGC and HR-TOFMS. Representative samples and analytes are shown.

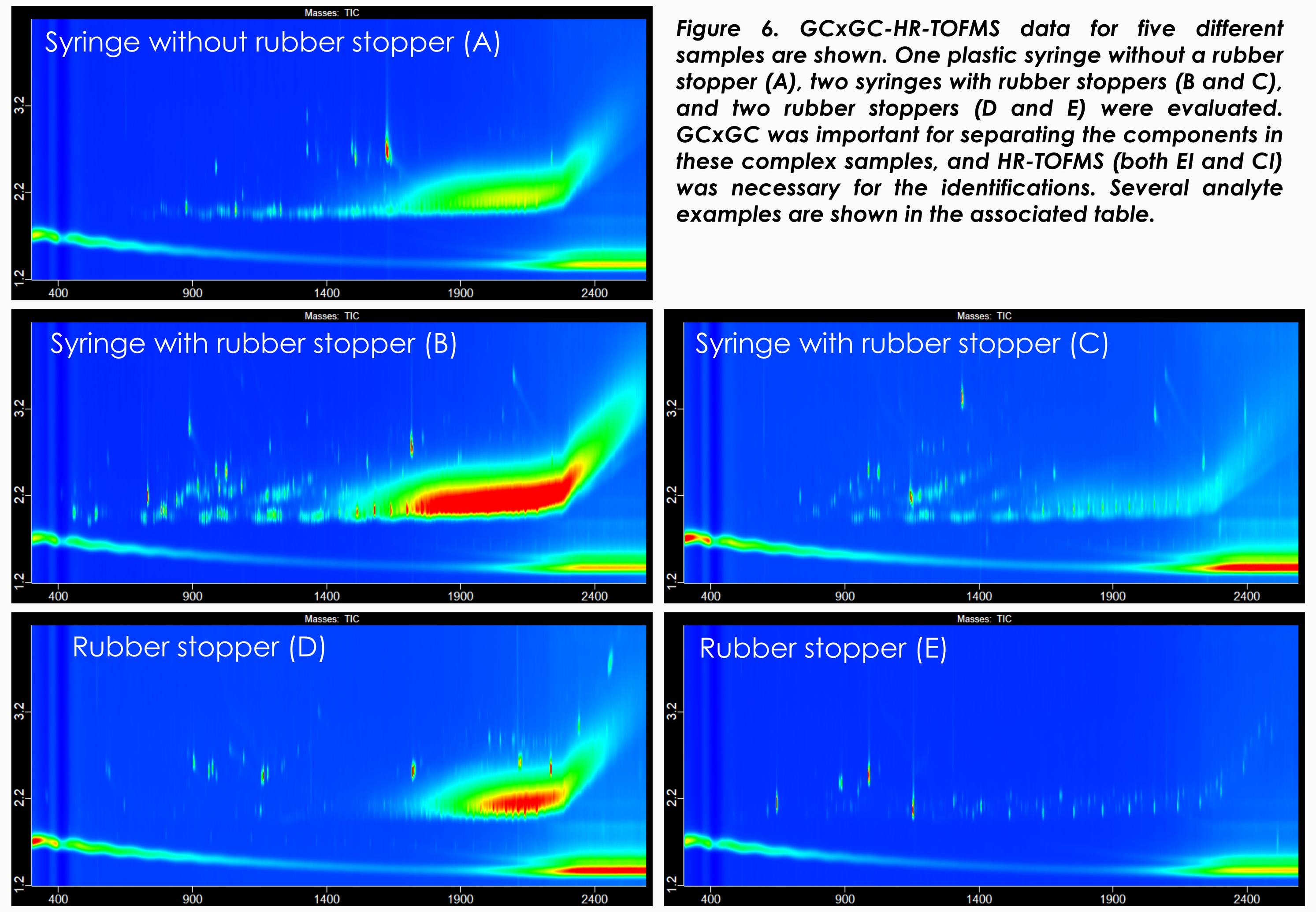

Figure 6. GCxGC-HR-TOFMS data for five different samples are shown. One plastic syringe without a rubber stopper (A), two syringes with rubber stoppers (B and C), and two rubber stoppers (D and E) were evaluated. GCxGC was important for separating the compounds in these complex samples, and HR-TOFMS (both EI and CI) was necessary for the identifications. Several analyte examples are shown in the associated table.

Analyte Name	Sim.	CAS	Formula	Obs Ion m/z	Exp. Mass	MA (ppm)	R.T. (s)	RI (Obs : Lib)	Notes	A	B	C	D	E
Triacetonamine	896	826-36-8	$C_9H_{17}NO$	155.13040	155.23772	-0.42	585, 2.616	1124: 1137	*stabilizer*		✓	✓		
Butyl Oligomer	NA		$C_{13}H_{24}$	180.18705	180.18725	-1.13	645, 2.152	1178 : NA	*butyl oligomer*		✓	✓	✓	✓
m-Di-tert-butylbenzene	937	1014-60-4	$C_{14}H_{22}$	190.17164	190.32500	0.22	732, 2.184	1261 : 1249	*polymer linking agent*	✓	✓	✓		
Chlorinated oligomer	NA		$C_{13}H_{23}Cl$	214.14822	214.14828	-0.30	879, 2.376	1404 : NA	*Halogenated oligomer*		✓	✓	✓	✓
4-tert-Pentylphenol	891	80-46-6	$C_{11}H_{16}O$	164.11948	164.24455	-0.54	882, 2.720	1408 : 1400	*antioxidant*		✓	✓	✓	
Chlorinated oligomer	NA		$C_{13}H_{23}Cl$	214.14809	214.14828	-0.87	888, 2.376	1414 : NA	*Halogenated oligomer*		✓	✓	✓	✓
Diphenyl ether	942	101-84-8	$C_{12}H_{10}O$	170.07252	170.20764	-0.59	888, 2.976	1414 : 1405			✓	✓		
Phenol, 2-chloro-4-tert-pentyl-	937	5323-65-9	$C_{11}H_{15}ClO$	198.08052	198.68955	-0.38	903, 2.624	1430 : 1460	*Phenol*		✓	✓	✓	✓
Phenol, 2-(1,1-dimethylethyl) - 4-ethyl-	907	96-70-8	$C_{12}H_{18}O$	178.13520	178.27117	-0.12	906, 2.568	1433 : 1459	*Phenol*		✓			
2,6-Di-tert-butylquinone	861	719-22-2	$C_{14}H_{20}O_2$	220.14592	220.30793	0.61	948, 2.384	1477: 1472		✓	✓	✓	✓	✓
2,4-Di-tert-butylphenol	940	96-76-4	$C_{14}H_{22}O$	206.16645	206.32440	-0.32	987, 2.489	1519 : 1514	*Antioxidant*	✓	✓	✓		
Butylated Hydroxytoluene	916	128-37-0	$C_{15}H_{24}O$	220.18214	220.35102	-0.12	990, 2.456	1522 : 1513	*Antioxidant*	✓	✓	✓	✓	✓
Isopropyl laurate	904	10233-13-3	$C_{15}H_{30}O_2$ +H	203.19044	242.39807	-0.36	1086, 2.176	1630 : 1618		✓	✓	✓		
Diphenylamine	866	122-39-4	$C_{12}H_{11}N$	169.08857	169.22288	-0.17	1104, 3.229	1651: 1622				✓		
Dodecyl acrylate	883	2156-97-0	$C_{15}H_{28}O_2$ +H	213.17592	240.38219	-0.97	1143, 2.200	1697 : 1697	*Acrylate*			✓		
Phenol, 2,4-di-t-butyl-6-nitro-	829	20039-94-5	$C_{14}H_{21}NO_3$	251.15175	251.32198	0.64	1143, 2.336	1697 : 1688	*Phenol*	✓	✓	✓	✓	
Butyl Oligomer	NA		$C_{21}H_{40}$	292.31211	292.31245	-1.18	1152, 2.072	1708 : NA	*butyl oligomer*				✓	✓
4-tert-Butyldiphenyl ether	890	5331-28-2	$C_{16}H_{18}O$	226.13520	226.31411	-0.05	1188, 2.768	1752 : NA	*Phenol*		✓	✓		
4-Formyl-2,6-di-tert-butylphenol	834	1620-98-0	$C_{15}H_{22}O_2$	234.16137	234.33454	-0.28	1209, 2.736	1778 : 1772	*Phenol*	✓	✓	✓	✓	✓
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	925	82304-66-3	$C_{17}H_{24}O_3$	276.17210	276.37130	0.37	1326, 2.696	1933 : 1923		✓	✓	✓	✓	
9,9-Dimethylacridan	926	6267-02-3	$C_{15}H_{15}N$	209.11982	209.28685	-0.39	1335, 3.328	1945 : NA				✓		
Metilox	759	6386-38-5	$C_{18}H_{28}O_3$	292.20331	292.41380	0.05	1341, 2.568	1953 : 1943	*plasticizer*	✓				
Dibutyl phthalate	908	84-74-2	$C_{16}H_{22}O_4$ +H	224.09991	278.34409	-1.97	1359, 2.752	1977 : 1965	*plasticizer*	✓	✓	✓		
Stearyl aldehyde	862	638-66-4	$C_{18}H_{36}O$ +H	269.28377	269.28389	-0.44	1395, 2.232	2027 : 2021			✓			
Palmitoleamide	904	106010-22-4	$C_{16}H_{31}NO$	253.23985	253.42405	-0.64	1494, 2.703	2172 : 2153		✓				
Hexadecanamide	877	629-54-9	$C_{16}H_{33}NO$	255.25574	255.43993	0.28	1506, 2.592	2190 : 2184		✓	✓			
N,N-Dimethyl palmitamide	823	3886-91-7	$C_{18}H_{37}NO$	283.28650	283.49316	-1.64	1554, 2.456	2265 : 2256		✓	✓	✓		
Oleamide	911	301-02-0	$C_{18}H_{35}NO$	281.27072	281.47728	-2.13	1626, 2.648	2381 : 2386	*slip agent*	✓				
Chemanox 22	916	88-24-4	$C_{25}H_{36}O_2$	368.27109	368.55307	0.29	1719, 2.752	2541 : 2529	*rubber antioxidant*		✓			
Irgafos 168	608	31570-04-4	$C_{42}H_{63}O_3P$	646.45084	646.92315	-0.15	2160, 2.429	3438 : 3397	*processing stabilizer*	✓				
Irganox 1076	838	2082-79-3	$C_{35}H_{62}O_3$	530.46981	530.86630	0.86	2235, 2.536	3620 : 2603	*antioxidant*	✓	✓	✓	✓	
Tris(2,4-di-tert-butylphenyl) phosphate	869	95906-11-9	$C_{42}H_{63}O_4P$	662.44656	662.92256	1.08	2238, 2.600	3628 : 3582	*Irgafos transformation product*	✓	✓	✓	✓	

CONCLUSIONS

In this work, GCxGC-HR-TOFMS was used to evaluate extracts from several pharmaceutically relevant materials. Two dimensions of separation helped address sample complexity, and high-resolution MS helped address analyte identification requirements. Several examples to highlight these benefits are shown, as well as representative samples and representative analytes.