Application Note

EMPOWERING RESULTS
Elemental Analysis | GC Mass Spectrometry | Metallography

Instrument: Pegasus® GC-HRT 4D

Characterization of Extractables from Common Pharmaceutical Packaging Materials with GCxGC and HR-TOFMS

Key Words: Extractable and Leachable, E&L, Pharmaceutical Materials, GCxGC, HR-TOFMS

Abstract

Comprehensive two-dimensional gas chromatography (GCxGC) and high-resolution time-of-flight mass spectrometry (HR-TOFMS) were used to characterize extracts from pharmaceutically relevant materials. Butyl rubber stoppers and plastic syringes were extracted with methylene chloride and subsequently analyzed with the Pegasus® GCxGC-HRT+4D, equipped with a Multi-Mode Ion Source™ (MMS™) (LECO Corporation, St. Joseph, MI, USA). The use of thermal modulation in combination with both a nonpolar and polar column, significantly increased separation selectivity and peak capacity, providing cleaner spectra for interpretation. HR-MS with both electron ionization and chemical ionization (EI and CI) provided spectra for commercial library searching and accurate mass data for formulae determinations and/or to support fragments and molecular ions. Chromatographic elution order in both dimensions—first dimension retention index (RI) and structured GCxGC chromatograms—was also used to support analyte identifications. Several representative materials were evaluated, and several representative analytes are highlighted.

Introduction

The characterization of extractable and leachable components in different types of materials and products is an important area of research. These studies enable insights into which chemicals (e.g., impurities, breakdown products, etc.) could be released from packaging or production materials (extractables) and what chemicals are released into products, such as pharmaceuticals or other consumer goods, which may have toxicological implications or concerns (leachables). Information about extractable and leachable components from packaging materials, delivery devices, and manufacturing equipment for pharmaceutical products is a particular area of growing interest and focus. Analytical testing to determine this information can also be part of submission requirements to the FDA or other regulatory bodies.

These analyses can be challenging due to the complexity of the samples and because there is the potential for many unknown features amongst the observed analytes. A variety of analytical methods can be used, and many approaches are compliant with testing guidance.[1] Generally, chromatographic separations coupled to mass spectrometry are needed to address both sample complexity and to determine tentative identifications.

In this work, we demonstrate the use of GCxGC and HR-TOFMS, with multiple ionization modes, to characterize the complex extracts. GCxGC enhances the chromatographic performance by separating the complex extracts with two stationary phases of contrasting polarities in a single analysis. This leads to an improved peak capacity, enhanced chromatographic separations, cleaner spectra, and provides additional context for identifications from the elution order. High-resolution MS detection, with EI and CI modes, enhances identification confidence by providing library searchable spectra, accurate mass, and molecular ion information for formulae support and/or determinations. Here, we highlight benefits and capabilities of this analytical approach, and show the analysis of representative samples and observed analytes.

Experimental

Extracts were prepared from various materials 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 hours. The extracts were then analyzed by GCxGC-HR-TOFMS (Pegasus HRT$^+$ 4D, LECO Corporation, St. Joseph, MI, USA), as described in Table I. Each sample was analyzed with both EI and CI (methane) using a *Multi-Mode Ionization Source (MMS)*. An alkane standard was also analyzed for retention index (RI) calculations.

Table I. Instrument (*Pegasus* GC-HRT$^+$ 4D) Conditions

Auto Sampler	**LECO L-PAL 3 Autosampler**
Injection	1 µL
Gas Chromatograph	**LECO GCxGC QuadJet™ Thermal Modulator**
Inlet	280 °C, splitless
Carrier Gas	He @ 1.4 mL/min, constant flow
Column	Primary: Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating Secondary: Rxi-17Sil MS, 0.9 m x 0.25 mm i.d. x 0.25 µm coating
Temperature Program	50 °C (hold 2 min), ramp 8 °C/min to 340 °C (hold 5 min) Secondary Oven: + 20 °C
Modulation	3 s with temperature maintained + 15 °C relative to secondary oven
Transfer Line	350 °C
Mass Spectrometer	**LECO *Pegasus* HRT**
Ion Source	LECO MMS - EI and CI (methane)
Ion Source Temperature	250 °C (EI) and 165 °C (CI)
Mass Range	35-900 m/z (EI) and 60-900 m/z (CI)
Acquisition Rate	125 spectra/s

Results and Discussion

Extracts from rubber and plastic materials associated with pharmaceutical products can be quite complex. These extracts contain a large number of analytes, including both known and unknown chemical features. Powerful analytical techniques are crucial for understanding these complex samples, and GCxGC with HR-MS is well-suited for this type of sample characterization. GCxGC chromatographically isolates more features, and HR-MS provides accurate mass data to assist with feature identification and understanding of these complex samples. Multiple complementary pieces of information are generated for each feature to support analyte identifications, or to help propose formulae and structures of complete unknowns. Individually, the enhanced chromatography and high resolution mass spectral data offer unique benefits for analysis of complex samples, and the combination of the two can take the analytical interpretation even further. An example of these capabilities is described in Figure 1.

GCxGC adds a second dimension of separation compared to single dimension GC, allowing significantly increased analyte separation to be achieved. Representative GC and GCxGC chromatograms for an extract of a plastic syringe with a rubber-tipped plunger are shown in Figure 1A. The GC data was collected with the same hardware and methods described in Table I, but with the modulator turned off. With GCxGC, the primary column effluent is modulated to a second column with a different polarity stationary phase where analytes are further separated, often allowing first dimension coelutions to be resolved. This leads to more chromatographically resolved analytes and cleaner MS spectra overall, enhancing identification ability. GCxGC also generates structured chromatograms where chemical compound classes elute in ordered bands across the GCxGC separation space, based on the difference in both analyte volatility and polarity, offering additional context to support identifications.

In some cases, the additional separation can uncover features that were obscured in the primary separation, as shown in Figure 1B, which highlights a small section of the separations shown in Figure 1A. The single GC separation determined a single analyte, tentatively identifying it as dodecyl acrylate. The GCxGC separation uncovered an additional feature, identifying this as 2,4-di-t-butyl-6-nitrophenol. The spectrum for the GC peak, shown in Figure 1C, had a good library match to dodecyl acrylate, but also contained some additional m/z that were not part of the library spectrum. The GCxGC data revealed that these additional m/z were from the second feature merged together with the primary peak in the GC data. The additional peak was chromatographically separated in the second dimension using GCxGC, generating cleaner mass spectral data, and therefore enabling tentative identifications of both features to be made with greater confidence. As shown in the peak table in Figure 1C, these identifications are supported by spectral matching to the NIST library (similarity scores of 883 and 829), and RI matching in the first dimension (RI observed of 1697 compared to library RI values of 1697 and 1688). The high-resolution data also supports identification further, providing the molecular ion for the phenol compound with a mass accuracy of 0.64 ppm. While a molecular ion was not observed for dodecyl acrylate in the EI data, mass fragmentation accuracies were good. With the *Multi-Mode Ion Source (MMS)*, additional CI data was readily collected and used to enhance and verify the molecular ions for both features, as shown in Figure 1D. The M+H adducts were observed with mass accuracies of -0.97 ppm and -0.95 ppm for dodecyl acrylate and the phenol compound, respectively.

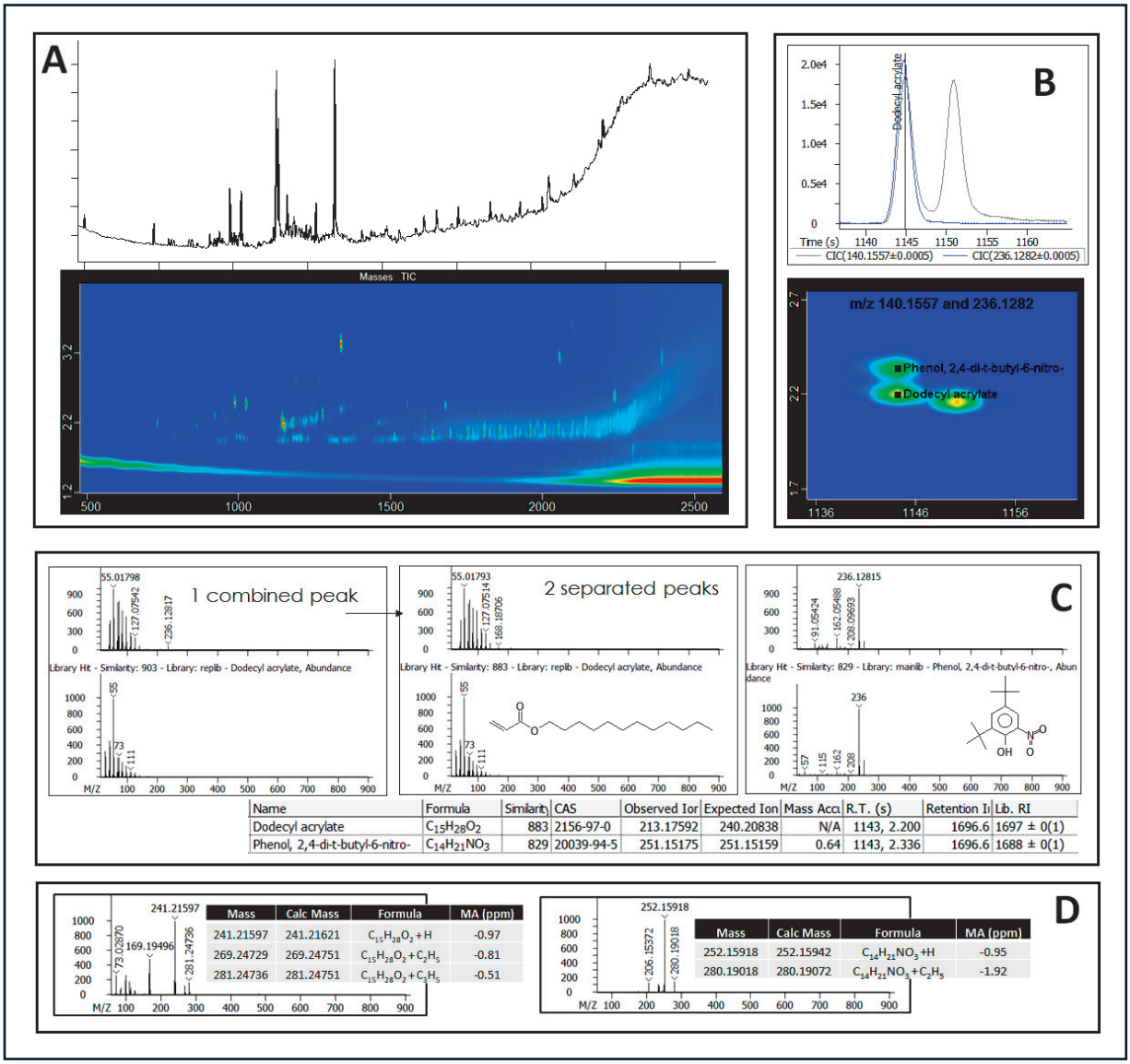

Figure 1. (A) Representative GC and GCxGC chromatograms for extracts from a plastic syringe with a rubber-tipped plunger. Analytes are separated in one dimension with GC and in two dimensions with the complementary stationary phases used for GCxGC. In some instances, GCxGC helps uncover new analytes that were hidden in the GC separation. (B) Here, a phenol compound and dodecyl acrylate completely coelute and are combined as one peak in the GC separation. Both were determined with GCxGC where the analytes were chromatographically separated in the second dimension. (C) The single peak true spectrum in the GC data is the combination of both peaks observed in the GCxGC data. Peak metrics like spectral similarity, first dimension RI, elution order in the structured GCxGC space, and mass accuracy supported these identifications. A molecular ion was observed for the phenol compound in the EI data also supporting the identification. (D) CI data added molecular ion information for dodecyl acrylate that did not have a molecular ion in the EI data and also supported the molecular ion for 2,4-di-t-butyl-6-nitrophenol. GCxGC revealed more information than could be determined with just GC.

As shown in Figure 1, GCxGC can uncover more features and HR-MS can then lead to tentative identifications of those features. Initial spectral matching to library databases typically provides the first proposed identification. The accurate mass data can then either support those identifications, as shown in Figure 1, or eliminate them as possibilities, as shown in Figure 2. The analyte shown in Figure 2 had a top library hit of an aromatic sulfur compound with a formula of $C_{13}H_{18}N_2S$, based on nominal mass spectral matching. However, this identification was not reasonable when comparing the observed accurate mass to the expected mass for that formula. If this were the formula for the observed molecular ion, the mass accuracy would be 182 ppm. The second library hit, with a formula of $C_{15}H_{22}O_2$, had a mass accuracy of -0.74 ppm. Switching from library hit #1 to library hit #2 provided a much-improved tentative identification, which also had support from spectral matching and RI.

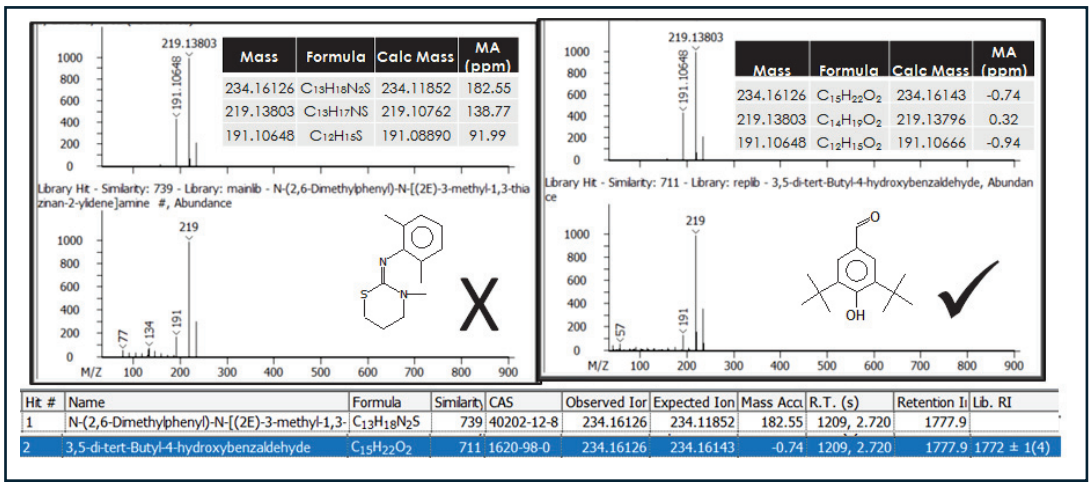

Figure 2. Accurate mass and formula determinations can also allow for selecting an improved spectral match from the library database. In this example from a plastic syringe extract, both library hit #1 and #2 were supported by nominal mass fragmentation patterns, but the formula for the first library hit was not well-supported and the second library hit was.

The information generated from this analysis can support or improve analyte identifications, as shown in Figures 1 and 2, and can also be used to better understand features when there is no preliminary library match, as shown in Figure 3. Four peaks (A, B, C, and D) from a rubber stopper extract are labeled in the chromatogram. These features did not have good spectral matches from the NIST library database. Thus, the observed accurate mass data were used to propose formulae. Feature A and B are supported with the formulae of $C_{13}H_{24}$ and $C_{21}H_{40}$ with mass accuracies of -1.13 ppm and -1.18 ppm, respectively. These are likely butyl rubber oligomers. Features C and D are likely chlorinated butyl rubber oligomers with formulae of $C_{13}H_{23}Cl$ with mass accuracies of -0.30 ppm and -0.87 ppm. In addition to the mass information, the chromatographic location of these features in the GCxGC contour plot adds support to the tentative identification. Furthermore, both butyl rubber oligomers and halogenated butyl rubber oligomers are anticipated analytes in rubber stopper extracts, which also adds support for these tentative identifications.

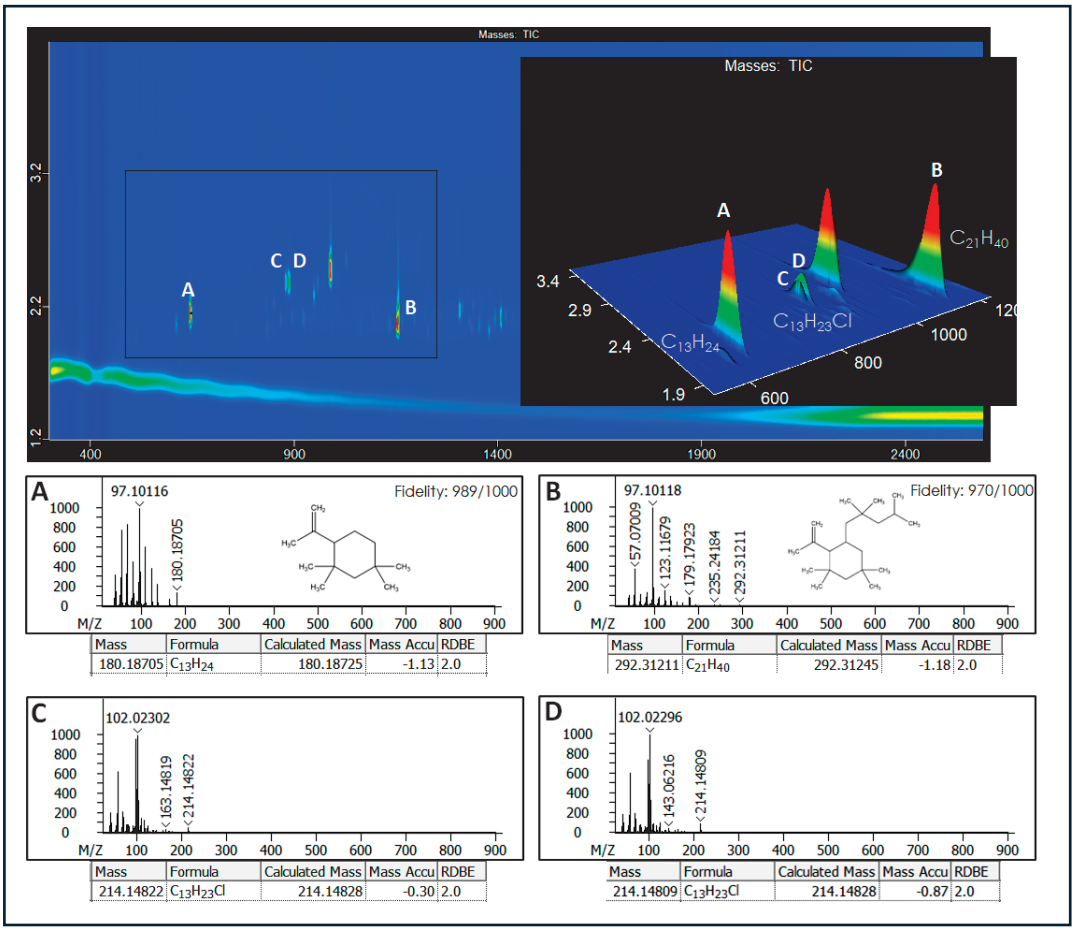

Figure 3. Accurate mass information can also help determine formulae for features that are not present in library databases. In this example, likely butyl rubber oligomers (A and B) and chlorinated butyl rubber oligomers (C and D) that were not in the NIST library database were observed in a rubber stopper extract. Accurate mass information was used to determine the formula. Elution position in the structured space and anticipated presence in these sample types also supported the tentative identifications.

The benefits and capabilities of this analytical approach allow for excellent characterization of complex samples like these extracts of pharmaceutical contact materials. As demonstrated in Figures 1-3, this analytical approach can isolate more individual analytes and provides data to support or propose identifications. A variety of sample extracts from plastic syringes, with and without rubber-tipped plungers and butyl rubber stoppers, were evaluated with GCxGC and HR-TOFMS. Representative GCxGC chromatograms are shown in Figure 4. Both the complexity and variation of the samples are apparent. GCxGC helped separate individual analytes from each other and from matrix background in the samples, and HR-MS helped to determine identifications for these features.

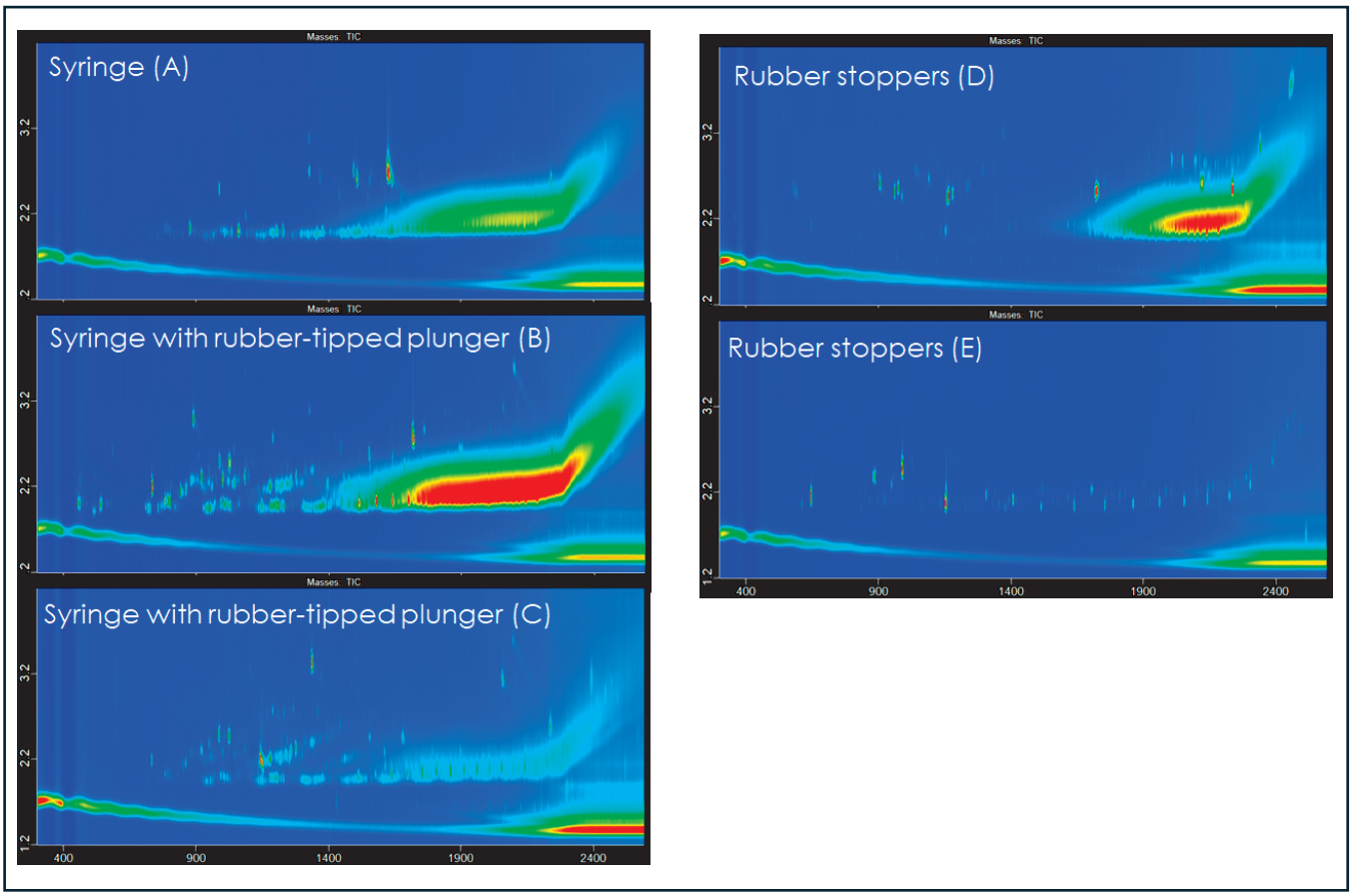

Figure 4. GCxGC-HR-TOFMS data for five different samples are shown. One plastic syringe without a rubber-tipped plunger (A), two syringes with rubber-tipped plungers (B and C), and two rubber stoppers (D and E) were evaluated. GCxGC was important for separating the components in these complex samples, and HR-TOFMS (with both EI and CI) was crucial for determining the identifications. Several analyte examples are shown in Table II.

There are many analytes of interest in these samples and some representative examples are listed in Table II. Support for these tentative identifications is listed in the table and can include spectral matching to library databases, matching accurate mass data for the molecular ion using EI and/or CI, library RI matching, and elution in the GCxGC structured space. The different materials had different extracts, as shown in Figure 4. Marks in columns A-E, corresponding to extracts from samples A-E, indicate whether that feature was observed in each sample.

presentative Analytes

ame	Similarity	CAS	Formula	Obs Ion m/z	Expected m/z	MA (ppm)	R.T. (s)	RI (Obs : Lib)	Notes	A
ne	896	826-36-8	$C_9H_{17}NO$	155.13040	155.23772	-0.42	585, 2.616	1124: 1137	*stabilizer*	
er	NA		$C_{13}H_{24}$	180.18705	180.18725	-1.13	645, 2.152	1178 : NA	*butyl oligomer*	
ylbenzene	937	1014-60-4	$C_{14}H_{22}$	190.17164	190.32500	0.22	732, 2.184	1261 : 1249	*polymer linking agent*	✓
oligomer	NA		$C_{13}H_{23}Cl$	214.14822	214.14828	-0.30	879, 2.376	1404 : NA	*halogenated oligomer*	
phenol	891	80-46-6	$C_{11}H_{16}O$	164.11948	164.24455	-0.54	882, 2.720	1408 : 1400	*antioxidant*	
oligomer	NA		$C_{13}H_{23}Cl$	214.14809	214.14828	-0.87	888, 2.376	1414 : NA	*halogenated oligomer*	
er	942	101-84-8	$C_{12}H_{10}O$	170.07252	170.20764	-0.59	888, 2.976	1414 : 1405		
oro-4-tert-pentyl-	937	5323-65-9	$C_{11}H_{15}ClO$	198.08052	198.68955	-0.38	903, 2.624	1430 : 1460	*phenol*	
-dimethylethyl) - 4-	907	96-70-8	$C_{12}H_{18}O$	178.13520	178.27117	-0.12	906, 2.568	1433 : 1459	*phenol*	
tylquinone	861	719-22-2	$C_{14}H_{20}O_2$	220.14592	220.30793	0.61	948, 2.384	1477: 1472		✓
tylphenol	940	96-76-4	$C_{14}H_{22}O$	206.16645	206.32440	-0.32	987, 2.489	1519 : 1514	*antioxidant*	✓
droxytoluene	916	128-37-0	$C_{15}H_{24}O$	220.18214	220.35102	-0.12	990, 2.456	1522 : 1513	*antioxidant*	✓
rate	904	10233-13-3	$C_{15}H_{30}O_2$+H	203.19044	242.39807	-0.36	1086, 2.176	1630 : 1618		✓
ne	866	122-39-4	$C_{12}H_{11}N$	169.08857	169.22288	-0.17	1104, 3.229	1651: 1622		
ylate	883	2156-97-0	$C_{15}H_{28}O_2$+H	213.17592	240.38219	-0.97	1143, 2.200	1697 : 1697	*acrylate*	
i-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*	✓
er	NA		$C_{21}H_{40}$	292.31211	292.31245	-1.18	1152, 2.072	1708 : NA	*butyl oligomer*	
phenyl ether	890	5331-28-2	$C_{16}H_{18}O$	226.13520	226.31411	-0.05	1188, 2.768	1752 : NA	*phenol*	
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*	✓
tyl-1-oxaspiro (4,5) ne-2,8-dione	925	82304-66-3	$C_{17}H_{24}O_3$	276.17210	276.37130	0.37	1326, 2.696	1933 : 1923		✓
acridan	926	6267-02-3	$C_{15}H_{15}N$	209.11982	209.28685	-0.39	1335, 3.328	1945 : NA		
	759	6386-38-5	$C_{18}H_{28}O_3$	292.20331	292.41380	0.05	1341, 2.568	1953 : 1943	*plasticizer*	✓
late	908	84-74-2	$C_{16}H_{22}O_4$+H	224.09991	278.34409	-1.97	1359, 2.752	1977 : 1965	*plasticizer*	✓
yde	862	638-66-4	$C_{18}H_{36}O$ +H	269.28377	269.28389	-0.44	1395, 2.232	2027 : 2021		
de	904	106010-22-4	$C_{16}H_{31}NO$	253.23985	253.42405	-0.64	1494, 2.703	2172 : 2153		✓
amide	877	629-54-9	$C_{16}H_{33}NO$	255.25574	255.43993	0.28	1506, 2.592	2190 : 2184		✓
palmitamide	823	3886-91-7	$C_{18}H_{37}NO$	283.28650	283.49316	-1.64	1554, 2.456	2265 : 2256		✓
	911	301-02-0	$C_{18}H_{35}NO$	281.27072	281.47728	-2.13	1626, 2.648	2381 : 2386	*slip agent*	✓
	916	88-24-4	$C_{25}H_{36}O_2$	368.27109	368.55307	0.29	1719, 2.752	2541 : 2529	*rubber antioxidant*	
	608	31570-04-4	$C_{42}H_{63}O_3P$	646.45084	646.92315	-0.15	2160, 2.429	3438 : 3397	*processing stabilizer*	✓
	838	2082-79-3	$C_{35}H_{62}O_3$	530.46981	530.86630	0.86	2235, 2.536	3620 : 2603	*antioxidant*	✓
-butylphenyl)	869	95906-11-9	$C_{42}H_{63}O_4P$	662.44656	662.92256	1.08	2238, 2.600	3628 : 3582	*Irgafos transformation product*	✓

Conclusion

In this work, GCxGC-HR-TOFMS was used to evaluate extracts from several pharmaceutically relevant packaging/delivery system materials. Two dimensions of separation helped address sample complexity and high-resolution MS helped address analyte identification requirements. GCxGC improved the peak capacity of the separation, which allowed for chromatographically isolating more analytes from each other and from interferences. This uncovered new analyte peaks, provided spectra with fewer interferences, and led to more identified analytes compared to one-dimensional GC. Incorporating EI and CI accurate m/z information from HR-TOFMS led to better identifications with more confidence. Analyte identifications were supported with spectral matching, accurate mass formulae, and GCxGC elution position. In situations without library matches, this data can help with proposing chemical formulae and structures. Several examples to highlight these benefits were shown, as well as representative samples and representative analytes.

References

[1]USP 1663 "Assessment of Extractables Associated with Pharmaceutical Packing/Delivery Systems"

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