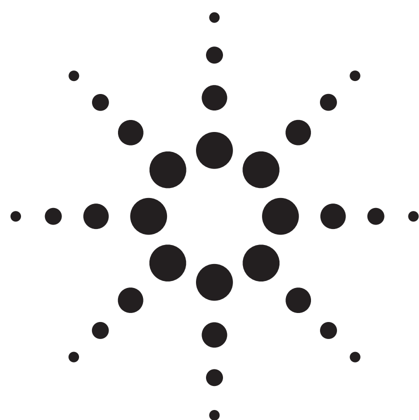

LC-TOF-MS As a Tool to Support Can Coating/Food Interaction Studies

Application

Food Safety

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Abstract

This application illustrates how time-of-flight mass spectrometry has been used in the studies of interactions at the can coating/food interface of internally coated metal cans intended for use in the food industry. Previously unconfirmed migrants were confidently identified using accurate mass information provided.

Introduction

The internal surface of metal cans used to pack foodstuffs is often coated to form a barrier between the food and the metal of the can. The coating formulation may contain various components, such as resins, cross-linking agents, catalysts, lubricants, wetting agents, and solvents. The potential exists for these ingredients, or by-products of reactions between them, to migrate from the can coating into foods.

Food ingredients such as fat or water can cause some coatings to swell, which may enhance any migration, particularly if the food is heat processed in its packaging. Migration can also depend on other factors: contact time and temperature, the type and thickness of the coating, and the molecular mass and size of the migrating species. Studies, in particular migration modeling, of interactions between the can coating and the foodstuff are important in understanding, and eventually reducing, migration of compounds from the can coating into the foodstuffs.

In previous applications we have described the analysis of can coatings based on epoxy resins [1] and polyester resins [2] and how the accurate mass information for the parent compounds and fragment ions greatly increased the confidence in the identification of unknown compounds. In this application we describe how liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS) was used as a tool to support studies on can coating/food interactions

Experimental

Coated Panels

The epoxyphenolic (EPH) lacquer was applied to metal panels and cured in an oven at 200 °C for 10 min. Small test specimens with an area of 9 cm^2 were cut from the panel and folded to a concertina shape ready for migration studies.

Exposure to Sunflower Oil

The folded test specimens of coated panel were submerged in sunflower oil in a pressurised vial at 121 °C in a silicon oil bath for 10, 20, 30, 40, 50, 60, 70, and 120 min. After exposure, the coated

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metal test specimens were wiped to remove the oil and submerged in acetonitrile (25 mL) overnight. A portion of the acetonitrile (1 mL) was passed through a 0.2-mm PTFE filter.

Hydrochlorination

Concentrated hydrochloric acid (100 µL) was added to a portion (500 µL) of the concentrated (10 times) EPH acetonitrile extract. This was allowed to react at 60 °C for 18 h in a sealed vial and made up to 1 mL with acetonitrile.

Liquid Chromatography-Fluorescence Detection (LC-FLD)

Instrument:	Agilent 1200 Series LC and G1321A FLD
Mobile phases:	A = 0.1% acetic acid in water B = acetonitrile
Gradient:	At t = 0, B = 35%; t = 5 min, B = 50%; t = 10 min, B = 50%; t = 20 min, B = 100%; t = 25 min, B = 100%
Flow rate:	1.0 mL/min
Column:	Agilent ZORBAX Eclipse XDB, 100 mm × 2.1 mm, 3.5-µm particle size (part number 961753.902)
Injection:	20 µL
Excitation wavelength:	275 nm
Emission wavelength:	305 nm
Gain:	2^10

LC-FLD-TOF-MS

Instrument:	Agilent 1200 Series LC, G1321A FLD and TOF positive electrospray
Mobile phases:	A = 0.1% acetic acid in water B = acetonitrile
Gradient:	At t = 0 min, B = 35%; t = 5 min, B = 50%; t = 20 min, B = 50%, t = 30 min, B = 100%; t = 40 min, B = 100%
Flow rate:	0.3 mL/min
Column:	Agilent ZORBAX Eclipse XDB, 100 mm × 2.1 mm, 3.5-µm particle size (part number 961753.902)
Injection:	5 µL
Excitation wavelength:	275 nm
Emission wavelength:	305 nm
Gain:	2^10
Nebulizer pressure:	30 psi
Capillary:	4000 V
Gas temperature:	325 °C
Drying gas:	10 L/min
Fragmentor:	150 V

Acylation

A method reported by Biedermann and Grob was adapted for use [3]. A portion (5 mL) of the EPH acetonitrile extract was evaporated to dryness under nitrogen. Acetic anhydride (25 µL) and pyridine (25 µL) were added and allowed to react for 15 min. The excess reagents were removed by evaporation under nitrogen and the residue was redissolved in acetonitrile (500 µL).

Another portion (5 mL) of EPH acetonitrile extract was evaporated under nitrogen and treated with acetic anhydride as described above. After removal of the excess reagents by evaporation, trifluoroacetic acid (TFAA, 100 µL) was added. This was allowed to react for 15 min and the excess reagent removed by evaporation under nitrogen. The dry residue was redissolved in acetonitrile (500 µL).

Results and Discussion

During migration tests using panels coated in a generic EPH coating, two co-eluting peaks were studied by LC-FLD. The identity of a pair of peaks at a similar retention time has been previously reported in the literature as two isomers of cyclo-di-BADGE [4]. The structure of cyclo-di-BADGE is shown in Figure 1.

The EPH coated panels were exposed to sunflower oil at 121 °C for increasing periods of time and the co-eluting peaks were seen to behave differently, as shown in Figure 2. The profile of the two peaks, 2-1 and 2-2, was seen to change: as the length of exposure increased, the relative peak height of peak 2-1 decreased compared to that of peak 2-2, suggesting that this compound was migrating at a greater

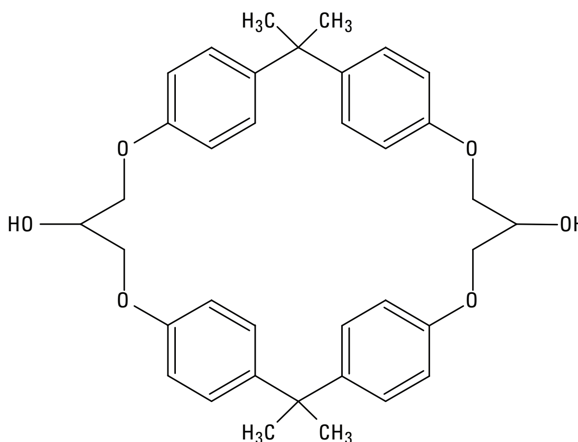

Figure 1. Structure of cyclo-di-BADGE, $C_{36}H_{40}O_6$.

rate. After 120 min, the two peaks had approximately equal peak heights.

The identification power of TOF-MS shown in previous applications [1-2] was used to investigate the EPH extracts with the aim of confidently identifying the co-eluting peaks and explaining the migration behavior seen. The flow rate of 1.0 mL/min used in the LC-FLD was deemed too high to be used on the LC-TOF-MS (although this ESI source can accept a flow rate of 1.0 mL/min), so the LC conditions were adapted. The LC-TOF-MS apparatus had a FLD in series. Figure 3 shows the FLD chromatogram of a concentrated acetonitrile EPH extract. The slower gradient means that the peaks are now eluting later (22.4 min and 23.15 min) and there are now three peaks, because the extra time on the column has allowed greater interactions with the stationary phase, which in turn has allowed greater resolution between the peaks.

This becomes clearer when looking at the TOF-MS data for this chromatogram (Figure 4). There are three peaks: 4-1 at 22.13 min, 4-2 at 22.61 min, and 4-3 at 23.45 min. Peaks 4-2 and 4-3 have the same mass spectra and molecular formula ($C_{36}H_{40}O_5$), as shown in Figure 5 and Table 1. Peak 4-1 has a different molecular formula ($C_{25}H_{34}O_5$).

From this data the molecular formula of peak 4-1 was proposed as $C_{25}H_{34}O_5$ with an identity of BADGE.BuOH. This identity was deduced based on methods reported in an earlier application [1]. The identity was confirmed by the addition of HCl to the extract. As expected, the BADGE.BuOH peak disappeared and was replaced by a peak with a

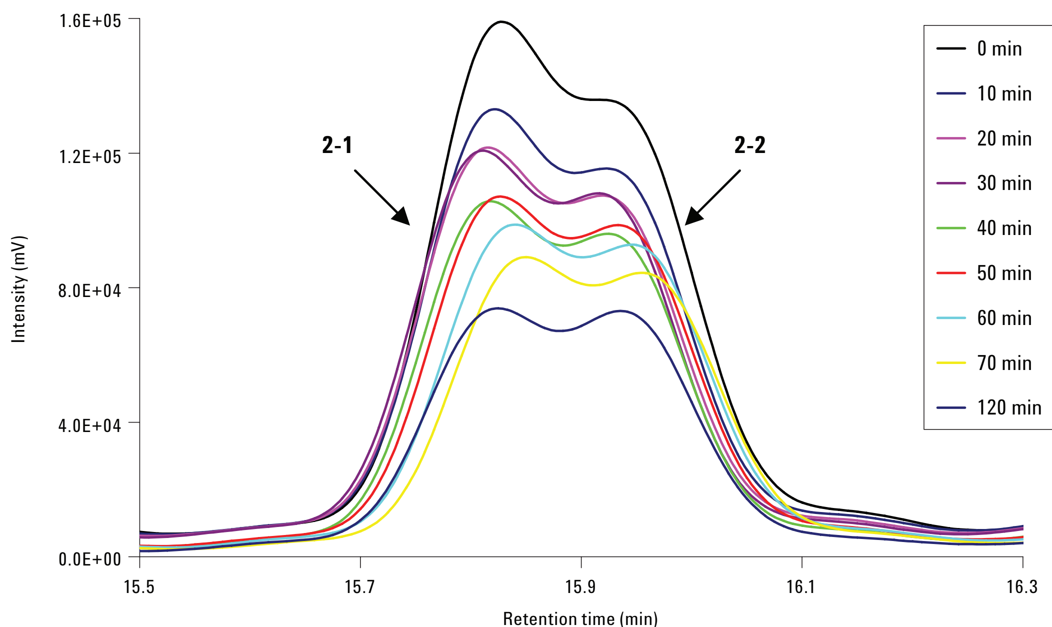

Figure 2. LC-FLD chromatogram obtained after exposing the EPH can coating to sunflower oil for different lengths of time.

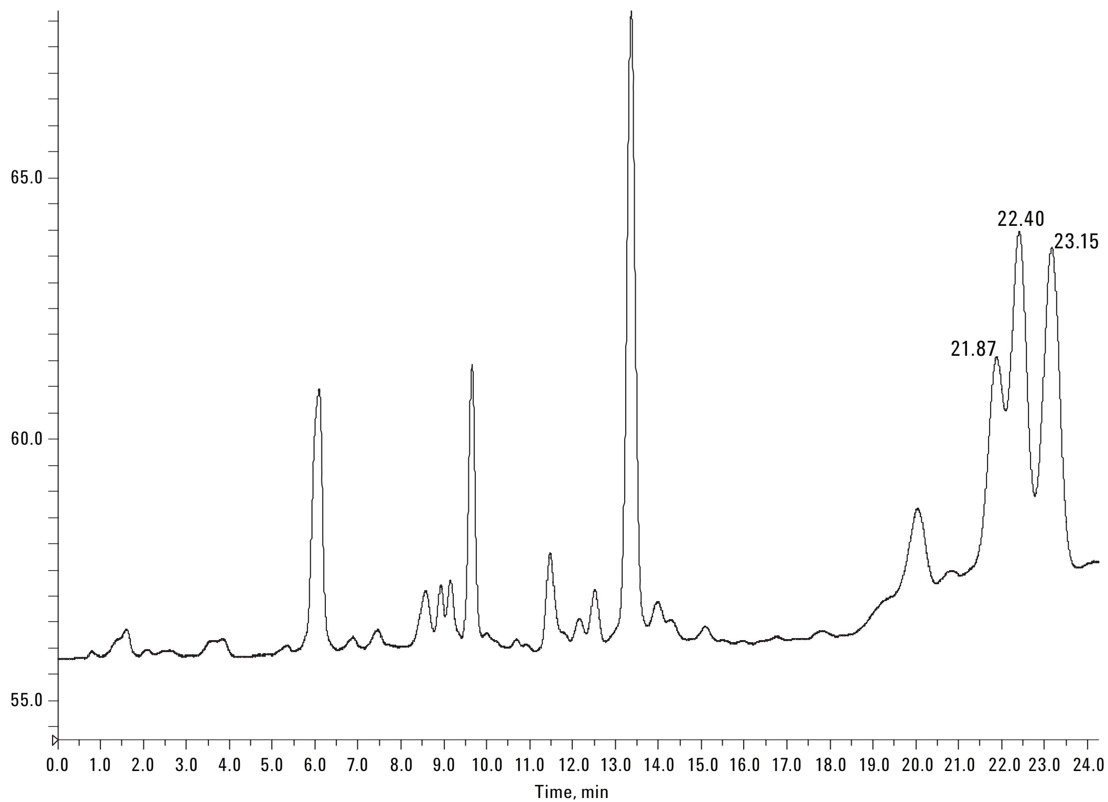

Figure 3. LC-FLD chromatogram of the concentrated EPH acetonitrile extract.

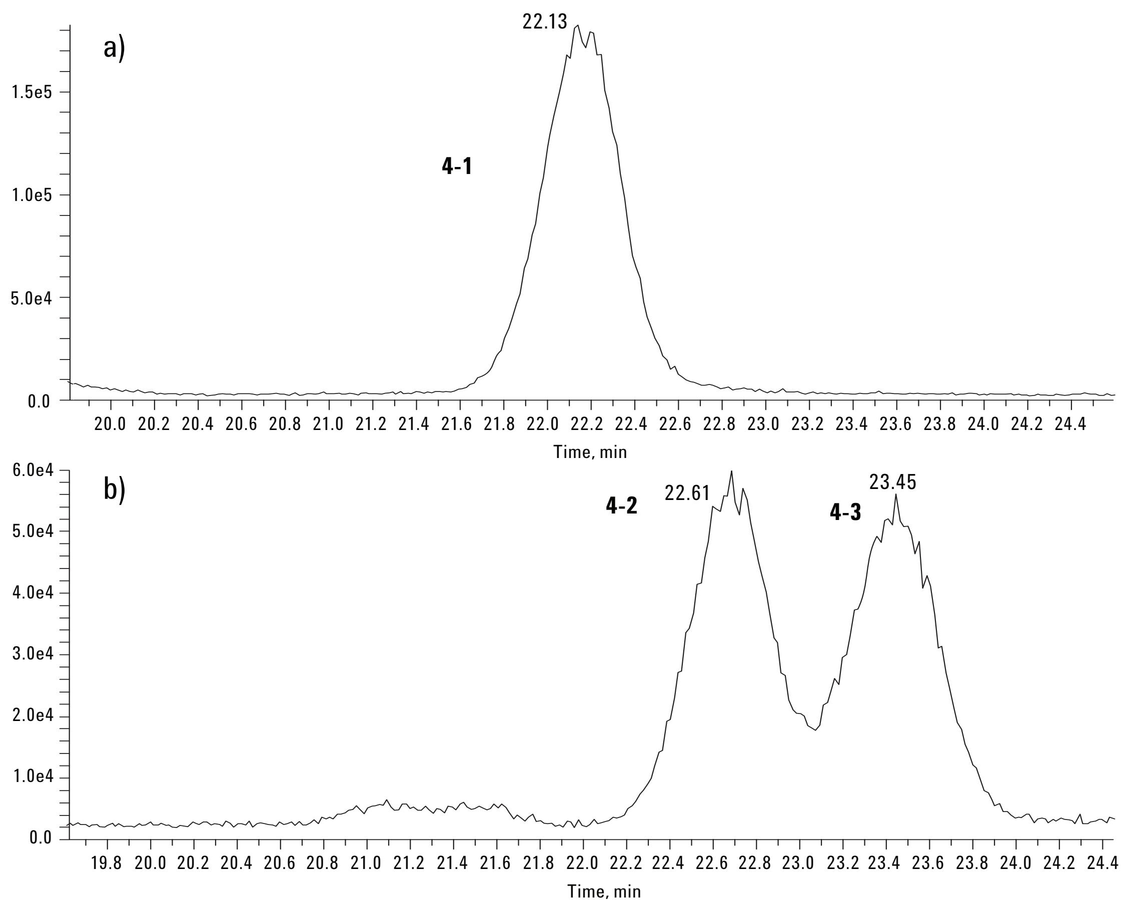

Figure 4. Extracted ion chromatograms for the three peaks of interest: a) *m/z* 436.98 - 437.48 and b) *m/z* 591.03 - 591.53.

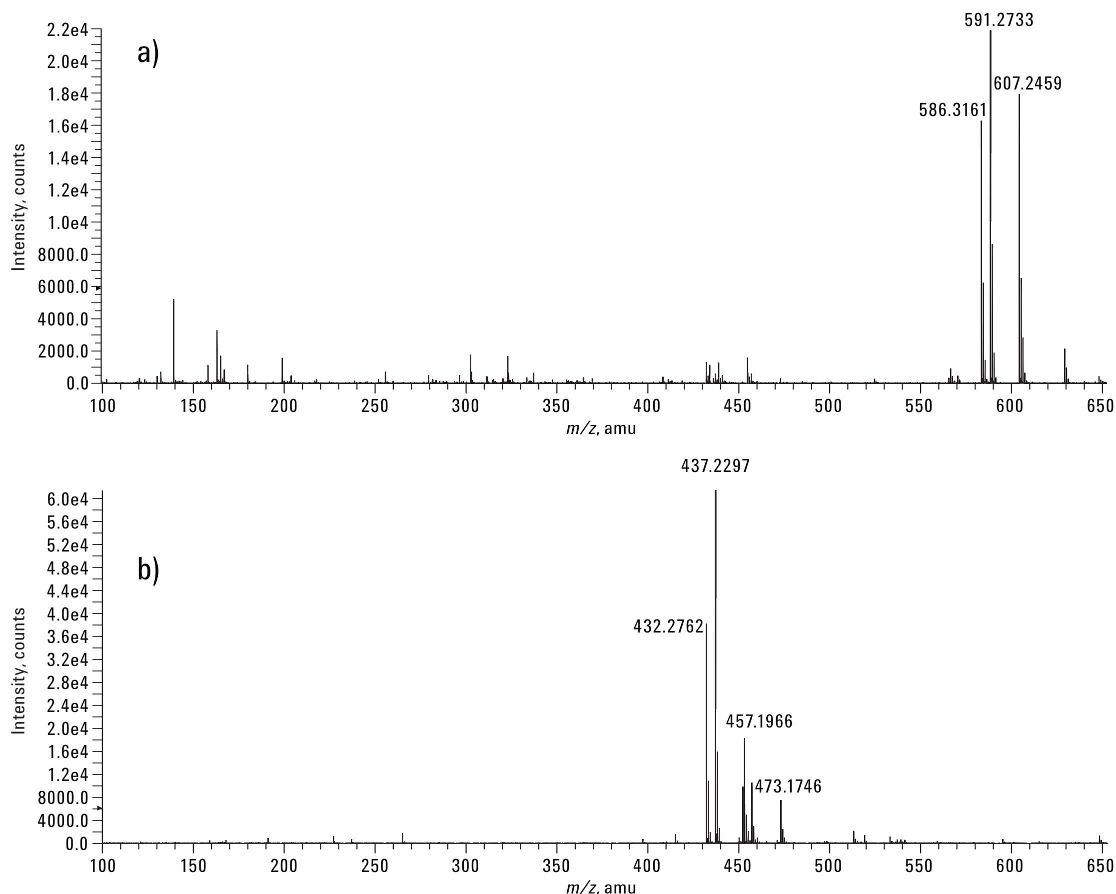

Figure 5. Mass spectrum of peak a) 4-1, b) 4-2, and 4-3.

Table 1. Molecular Formula Database Information for Peaks 4-1, 4-2 and 4-3

Peak	Mass	Molecular formula predicted	Theoretical mass	Mass error (PPM)	Molecular adduct
4-1	432.2746	$C_{25}H_{38}NO_5$	432.2744	0.12	$M+NH_4$
	437.2300	$C_{25}H_{34}O_5Na$	437.2298	0.35	M+Na
4-2	586.3163	$C_{36}H_{44}NO_6$	586.3163	–0.026	$M+NH_4$
	591.2722	$C_{36}H_{40}O_6Na$	591.2717	0.83	M+Na
4-3	586.3161	$C_{36}H_{44}NO_6$	586.3163	–0.37	$M+NH_4$
	591.2725	$C_{36}H_{40}O_6Na$	591.2717	1.3	M+Na

molecular formula consistent with that of BADGE.BuOH.HCl, as the HCl adds across the remaining epoxide ring. Figure 6 shows the relevant extracted ion chromatograms. Figure 7 shows the mass spectrum of BADGE.BuOH.HCl, with excellent correlation between experimental and theoretical chlorine isotope patterns.

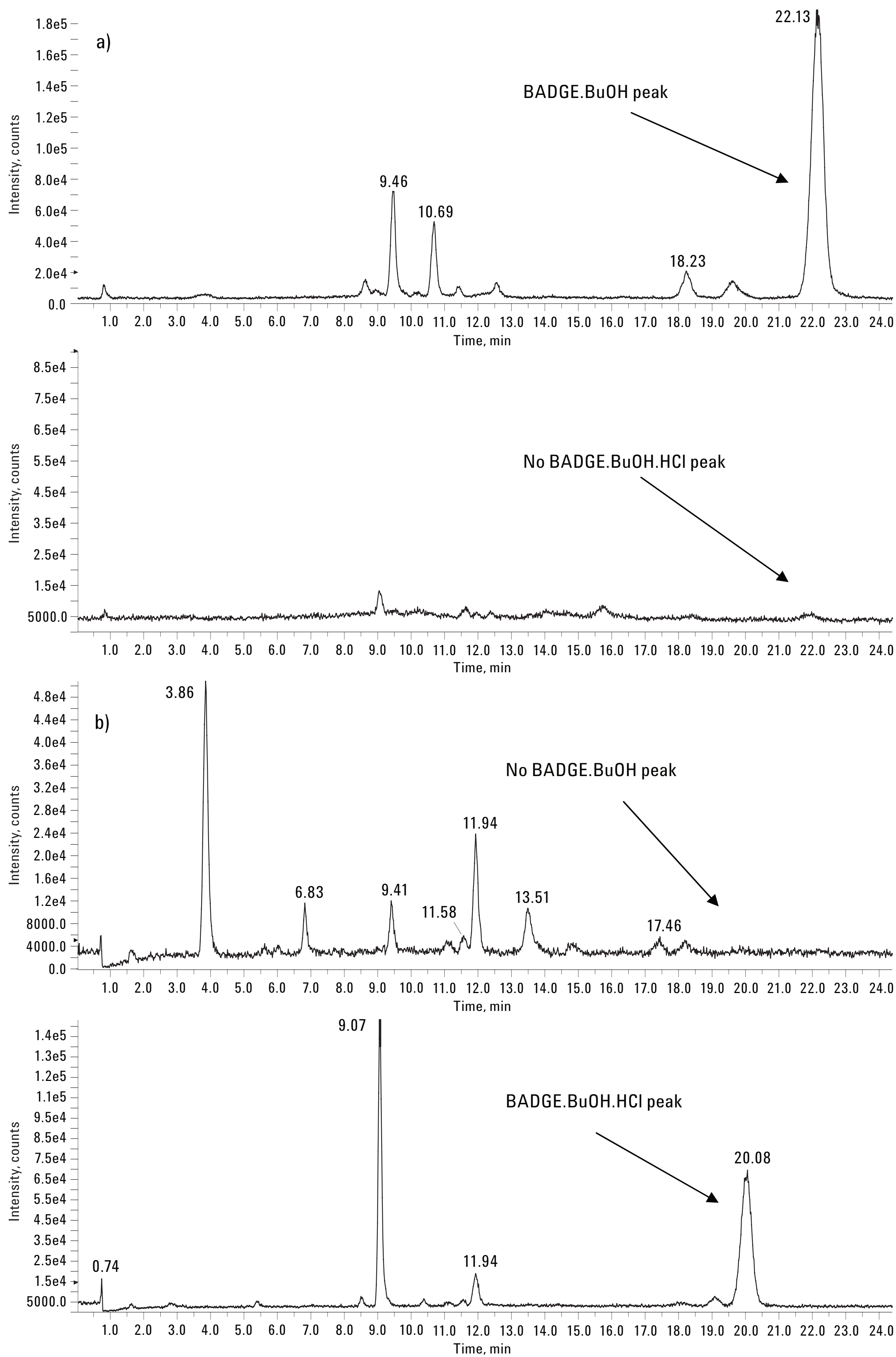

Figure 6. Extracted ion chromatograms for BADGE.BuOH (*m/z* 437 – 438) and BADGE.BuOH.HCl (*m/z* 451 – 452) for a) untreated EPH extract and b) EPH extract treated with HCl.

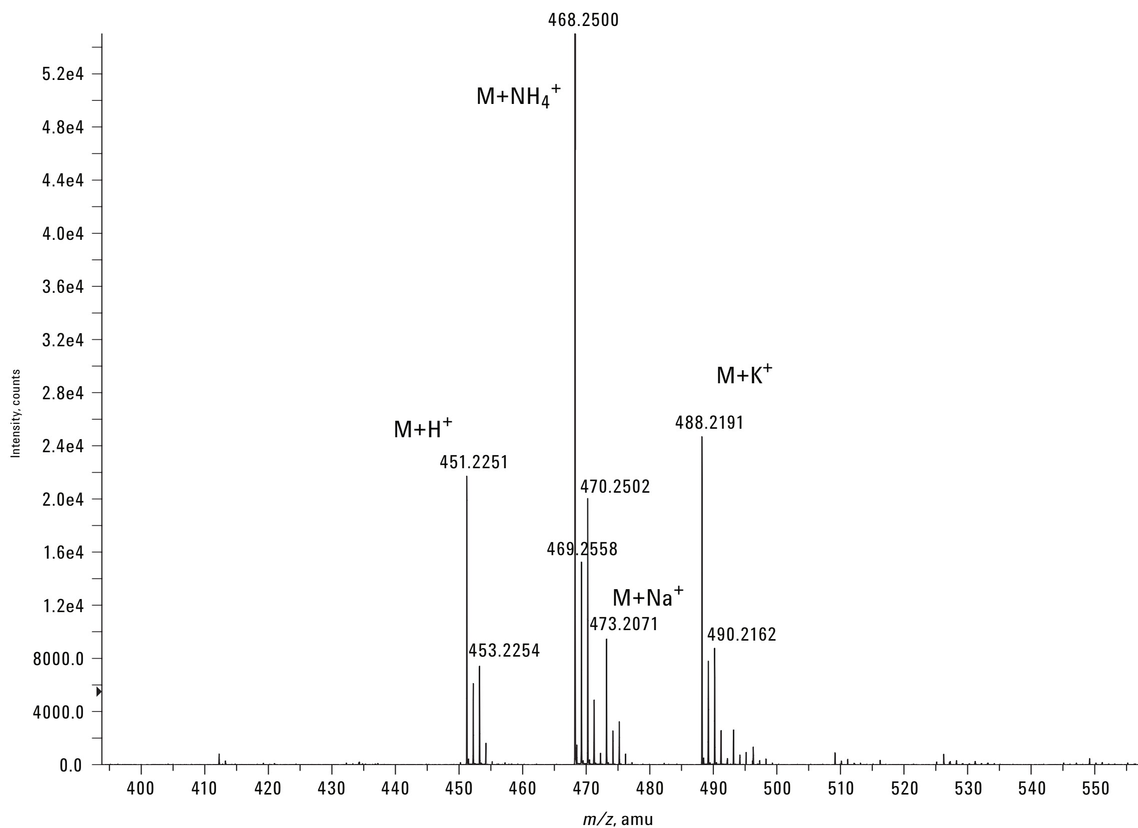

Figure 7. Mass spectrum of the peak at 20.1 min, corresponding to BADGE.BuOH.HCl.

Peaks 4-2 and 4-3 had the same mass spectra and were proposed to be $C_{36}H_{40}O_6$. As well as corresponding to cyclo-di-BADGE as suggested above, this could also correspond to BADGE.BPA, a linear BADGE derivative with the same molecular formula (see Figure 8).

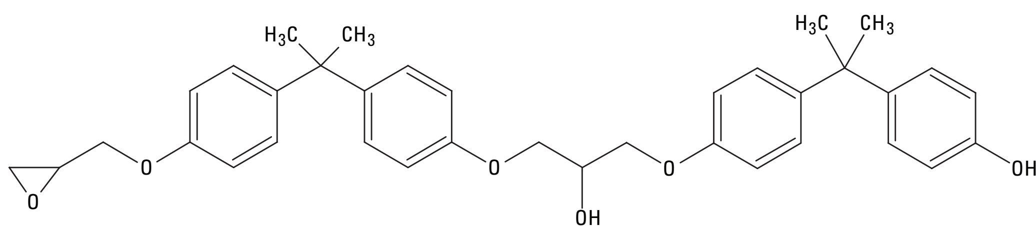

Figure 8. Structure of BADGE.BPA, $C_{36}H_{40}O_6$.

The addition of HCl did not change the two co-eluting peaks, which suggests that they are in fact due to cyclo-di-BADGE and not BADGE.BPA, as any peaks due to BADGE.BPA would be expected to disappear as the HCl will add across the epoxide ring. This conclusion was tested further; one of the differences between the proposed structures is that cyclo-di-BADGE has two hydroxide groups but BADGE.BPA has additional epoxide functionality as well as two hydroxide groups. It has been reported that acetic anhydride and TFAA react differentially with hydroxide and epoxide groups [3]. Acetic anhydride causes acylation of free hydroxide groups while further addition of TFAA causes acylation across the epoxide ring (see Figure 9). LC-TOF-MS analysis of the EPH extract after treatment with acetic anhydride showed the presence of a doubly acylated compound, either compound 9-1 or 9-2 ($C_{40}H_{44}O_8$). After further addition of TFAA the absence of a peak corresponding to compound 9-3 suggests that the two co-eluting peaks are in fact both due to cyclo-di-BADGE. The reason for two peaks is proposed to be because of the presence of stereoisomers, with the two hydroxide groups being cis- or trans- to each other, depending upon the side from which the phenol group attacks the epoxide ring during formation [3].

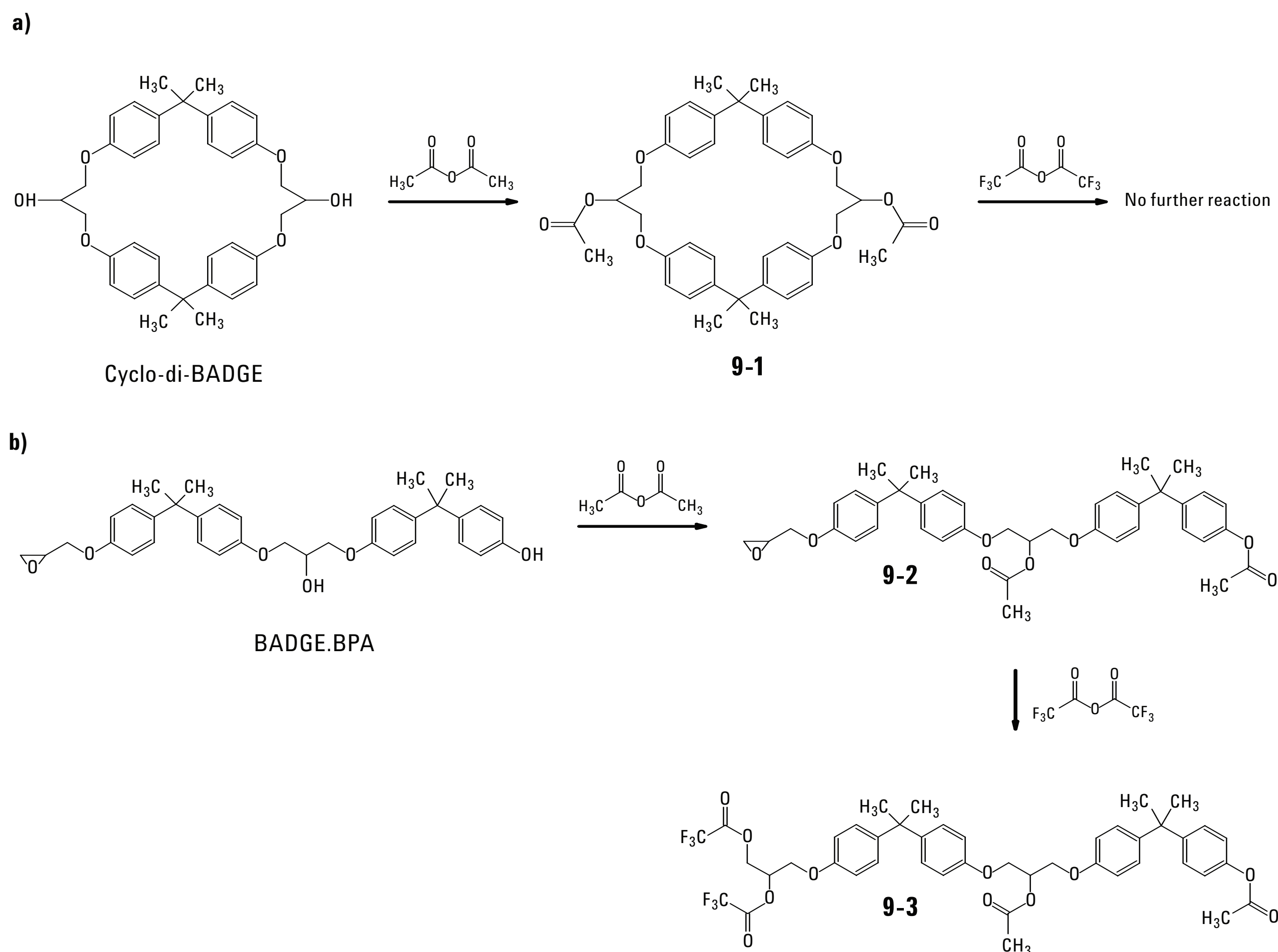

Figure 9. Acylation reactions for a) cyclo-di-BADGE and b) BADGE.BPA.

Conclusions

During migration studies at Leeds University, two co-eluting peaks were seen to behave differently during exposure to sunflower oil at different temperatures. There were in fact three co-eluting peaks and these were identified using LC-TOF-MS. The first peak was identified as BADGE.BuOH, and the second and third peaks were confirmed as *cis*- and *trans*- isomers of cyclo-di-BADGE. It is suggested that the differences seen in the original migration studies were due to the differences in structure between BADGE.BuOH and cyclo-di-BADGE, with the BADGE.BuOH migrating faster than cyclo-di-BADGE into the simulants, and the two stereoisomers of cyclo-di-BADGE migrating at the same rate as each other, which would account for the changes in peak height.

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