Applikationsbericht

A New Strategy for Confident Characterization of Extractables from Post-consumer Recycled Plastics Using LC-QTof

Hania Khoury-Hollins

Waters Corporation

Abstract

Plastic is ubiquitously present in every aspect of our modern lives. Plastic recycling is a way of preserving our natural resources and reducing our environmental footprint. Successful recycling implies that recycled plastics ought to have similar properties to its virgin equivalent. In addition, it needs to comply with the different consumer safety requirements. In this study, a discovery workflow was applied using liquid chromatography coupled to high resolution time of flight mass spectrometry to analyzse post-consumer recycled (PCR) low density polyethylene (LDPE). Key marker compounds were identified in various LDPE samples that belonged to different recycled LDPE batches.

Benefits

- Distinguishing different qualities of post-consumer recycled material (PCR)
- Confident identification of compounds linked to the quality and physical properties of recycled PCR using the power of high-resolution accurate mass measurements delivered by the Xevo™ G3 QTof technology
- Tool for investigation of PCR product safety for its re-introduction into the economy
- One integrated, streamlined instrument, and software solution with Xevo G3 QTof and MassLynx™ and Progenesis™ QI

Introduction

Most of the produced plastic is made from fossil fuels [https://www.ciel.org/issue/fossil-fuels-plastic/].[1] Plastic has become an essential part of our modern life. It can be found in medical equipment, household tools, packaging, ***etc.*** Consequently, it constitutes a big portion of household waste. Because of its properties, fossil-fuel derived plastic can remain intact for decades or even centuries after use. Therefore, decades of use of short-lived, single use plastic led to a global environmental catastrophe.[2] [UN plastic general assembly https://www.un.org/pga/73/plastics/].

In order to preserve our natural resources and reduce the environmental impact of plastic production and waste, we need to consider plastic waste as a valuable resource. By recycling we can reduce the dependence on fossil fuel for plastic production and tackle the plastic waste problem by keeping it in circulation for as long as possible.

To successfully reintroduce or repurpose post-consumer recycled plastics (PCR), there is a need to identify impurities that may affect its performance and imperil consumer safety. High levels of contamination originating from plastics' life cycle could restrict its prospective use. Therefore, it becomes the burden of the recycling industry to investigate the quality of PCR produced not only for its physical properties, but as well to identify and quantify any harmful chemical content that may threaten the safety of the user. The strictness of safety criteria varies depending on the potential end-use. Food contact material (FCM) for instance have very low tolerance threshold for chemicals such as phthalates, bisphenols, and mineral oil hydrocarbons compared with outdoor furniture made from recycled plastics. Thus, there is a need for a flexible workflow allowing the detection and identification of impurities in different batches of PCR.

Discovery workflow is an important unbiased analytical approach frequently used to characterize changes between different conditions. It is widely utilised in systems biology studies and has recently been applied to the identification of migrants coming from plastic food packaging material [Martinez-Bueno ***et al***. 2019].[3] The analysis is typically performed using liquid chromatography (LC) or gas chromatography (GC) coupled to high resolution mass spectrometry (HRMS).

In this application note, a novel strategy was employed for the analysis of different batches of recycled LDPE (rLDPE). Using the ACQUITY™ Premier LC coupled to Xevo G3 QTof the non-volatile migrant compounds from different batches of rLDPE were analyzsed. The data was then directly processed using Progenesis QI™ software.

Differences between qualities of rLDPE were observed and differentially expressed compounds were annotated using Food Contact Compounds (FCCdb) [Groh ***et al.*** 2021] and Chemicals associated with Plastic Packaging databases (CPPdb) [Groh ***et al*** 2019].[4–5] The annotated compounds were identified and confirmed using commercially available chemical standards.

Experimental

Sample Preparation

Four groups of samples were supplied by an industrial supplier of recycled material. Virgin LDPE (vLDPE), virgin LDPE with additives (vLDPE+), poor-quality recycled LDPE (poor rLDPE), and good-quality recycled LDPE (good rLDPE). Extracts from different groups were prepared as following: 15 g of pellets or 2.5 g of fluff were immersed in 100 mL of methanol. The extraction is carried out at 40 °C for 1 hour under magnetic stirring. The container is kept closed to avoid solvent evaporation. Methanol was then collected and filtered using 0.45 micron PVDF filters.

After preparing the extract from virgin plastic pellets and different qualities of rLDPE, a quality control sample (QC) was prepared by mixing 100 µL of each of the extracts. Samples and QC were then analyzed using reverse phase chromatography followed by high resolution mass spectrometry. Each sample was injected twice. The order of injection was randomized, and a QC was injected in between five sample injections and at the beginning and end of the sequence.

LC-MS Conditions

LC system:	Waters™ ACQUITY Premier Liquid Chromatography System
Vials:	LC-MS certified clear glass (p/n: 600000671CV)
Column:	CORTECS™® C_{18}, 1.6 µm, 2.1 x 100 mm, 90 Å Column (p/n: 186007095)

Column temperature:	50°C
Sample temperature:	6°C
Injection volume:	5 µL
Flow rate:	0.3 mL/min
Mobile phase A:	H_2O + 0.1% acetic acid (v/v)
Mobile phase B:	100% Methanol

Gradient Table

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0	0.3	98	2	Initial
0.5	0.3	98	2	6
8.5	0.3	2	98	6
13.5	0.3	2	98	6
13.5	0.3	98	2	1
16	0.3	98	2	1

MS Conditions

MS system:	Xevo G3 QTof

Source conditions:	Desolvation temperature: 600°C Desolvation gas (L/h): 600 Source temperature: 150°C Cone Gas (L/h): 150 Source offset: 80 Sampling cone: 30
Capillary voltage:	1 kV
Ionization mode:	ESI +/-
Mass range:	*m/z* 50–1200
Acquisition rate:	5 spectra per second (Hz)
Lock mass:	Leucine enkephalin (*m/z* 556.276 and *m/z* 554.262, for positive and negative modes, respectively). Lock mass spectrum acquired
Acquisition mode:	High Definition MS^E ($HDMS^E$) a data independent acquisition method
Collision energy:	Low collision energy: 0 High collision energy: ramp (20 to 40 eV/20 to 50 eV for positive and negative modes, respectively).

Software Tools

Data acquisition was performed using MassLynx™ v4.2 and the statistical analysis was performed using Progenesis™ QI 3.0.7929.47290

Results and Discussion

Discovery workflows are well described in systems biology. We have applied a similar approach for the characterization of different post-consumer recycled plastics (PCR) [Broadhurst *et al.*, 2018].[6] Briefly, samples and QC were analyzsed by LC-MS, the acquired raw data was then processed by Progenesis QI. The peak picking was performed by deconvoluting the detected ions and grouping the adducts when present into one compound. For the positive mode protonated and sodiated molecular ions were grouped as one compound. And for the negative mode of ionization, the deprotonated and acetate adduct were grouped as one compound. After a mass and retention time alignment step, the data was normalized to all compounds.

Out of 33 injections in positive mode 13816 and 7955 compounds were detected in positive and negative modes, respectively. Multivariate analysis allowed us to remove the noise and narrow down the list into compounds with a *p*-value ≤0.05 (Figure 1).

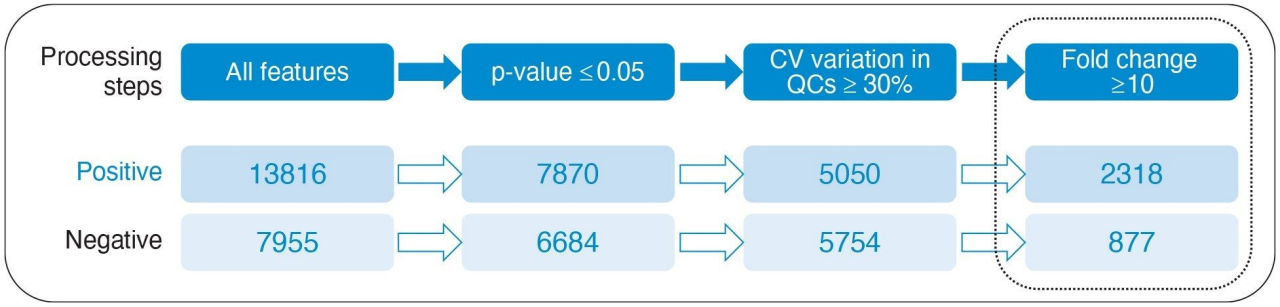

Figure 1. Summary of the data processing steps and the resulting number of compounds in positive and negative modes. The data analysis was performed using Progenesis QI.

To narrow down further the list of potential compounds, we have excluded any of the detected compounds having a coefficient of variation (CV) ≥30% in the quality control injections (QC). As a result, the compound list was reduced to 5050 and 5754 in positive and negative modes, respectively (Figure 1). Finally, we have retained the list of compounds that showed more than 10-fold change (FC) compared to the Virgin pellet extracts. [Martinez-Bueno *et al*. 2019]. [3] This final list of compounds is then used in all the following statistical analysis.

To investigate the relation between the analyzed samples we have performed principal component analysis

(PCA), a non-parametric approach to help visualising the relation between the samples (Figure 2).

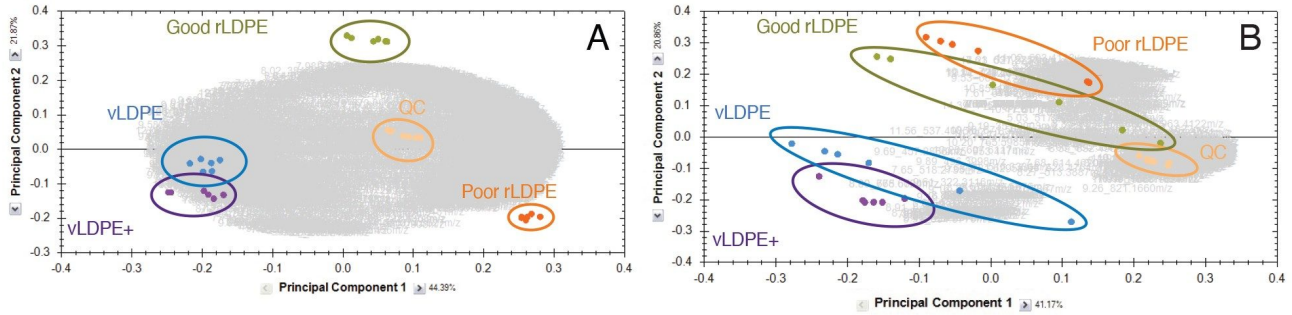

Figure 2. Principal component analysis (PCA) for the positive mode (A) and in the negative mode (B) compounds.

Figure 2 depicts a scatter plot of the first and second PCA components that capture the maximum variation in the data. Components 1 and 2 are 44.39% and 21.87% for the positive mode and 41.17% and 20.86% for the negative mode data, respectively.

In both the positive and negative modes (Figure 2A and 2B), the QC injections form a tight cluster. Consequently, any observed grouped cluster of samples can be interpreted as a similarity between the samples rather than a random event.

In both modes of ionisation, the virgin pellets (vLDPE) and the virgin pellets with additives (vLDPE+) form two analogous group (Figure 2A and 2B). Indeed, these two samples are very similar except for the few additives in the latter. The additives give a slight distinction between the two groups in the positive mode (Figure 2A). However, vLDPE and vLDPE+ cannot be significantly distinguished in positive and negatives modes of ionisation. On the other hand, there is a clear distinction between vLDPE (with or without additives) and the good and poor-quality rLDPE (Figure 2A and 2B). The distinction between the subgroups is more prominent in the positive mode of ionization where the poor-quality rLDPE extracts were easily distinguished from the good-quality rLDPE extracts (Figure 2A). The negative mode data is more scattered, and the poor and good-quality rLDPE cannot be significantly distinguished. Hence, the compounds detected in the positive are more critical to separate the two groups of rLDPE extracts from each other and from the virgin pellets.

To identify significant marker compounds in each group a correlation analysis on the 2318 compounds detected in positive was performed. Correlation analysis points out different groups of compounds with similar abundance tendencies. The dendrogram in figure 3 illustrates the different compounds detected in positive mode. Each

branch of the tree corresponds to a group of compounds with similar abundance trends (Figure 3, top panel). The *y*-axis corresponds to the distance between the tree branches. The smaller the distance value, the stronger the correlation between the compounds. A threshold of 1 is indicated on the figure. The arrow points out two at branches of compounds with strong correlation value of less than 0.85. The selected two compound branches display similar abundance profile (Figure 3 bottom panel). These compounds are unique or highly abundant in the poor-quality rLDPE. These compounds are also detected in the QC samples at a lower abundance due to the dilution factor when preparing QC samples.

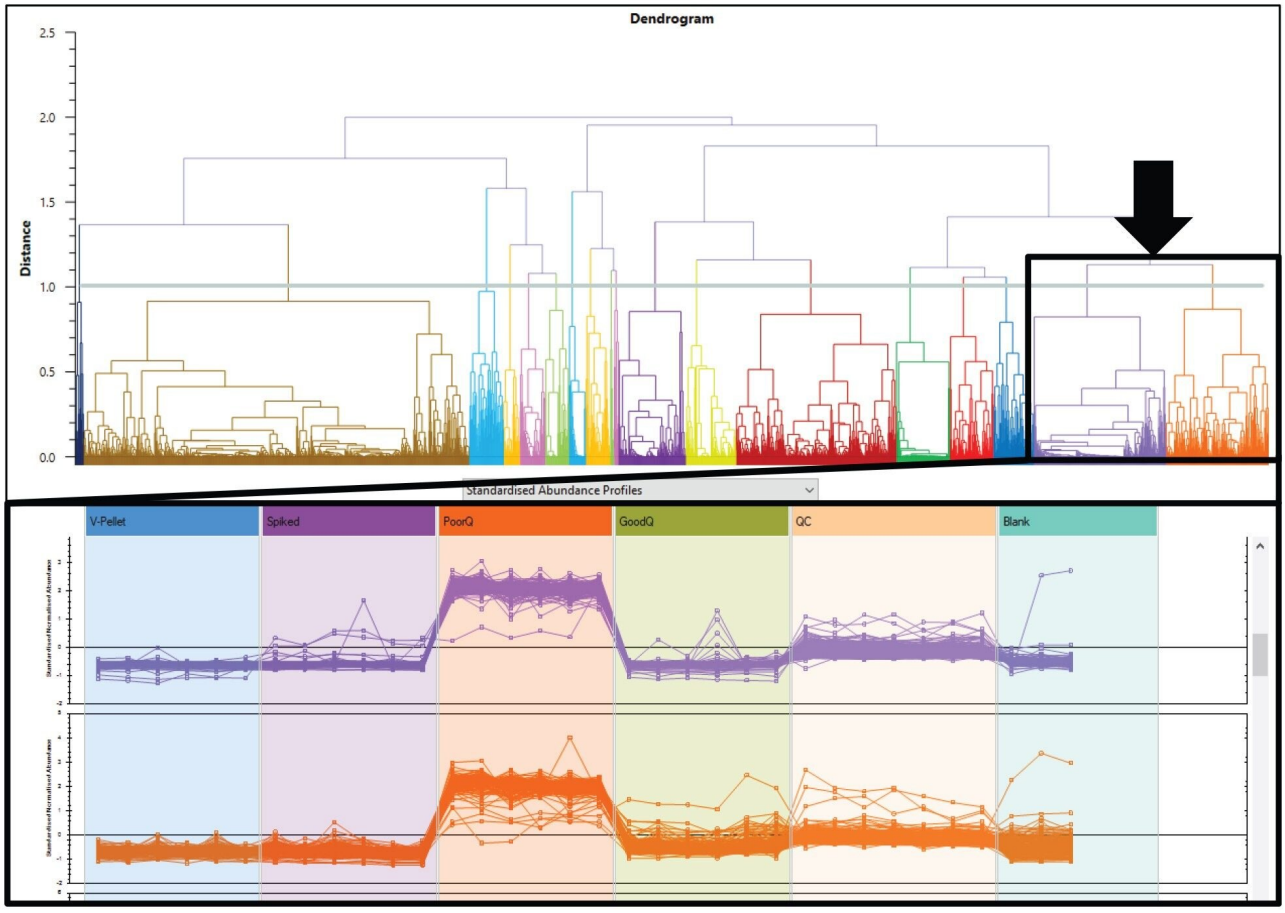

Figure 3. Correlation analysis between the detected compounds in the positive mode of ionisation. Top panel corresponds to a dendrogram with a threshold distance selected at 1.0. Bottom panel illustrates the abundances of the compounds within the same tree branch in the different sample injections. Each sample group is distinguished by a different column colour. From left to right: Blue: vLDPE, Violet: vLDPE with additive, Orange: poor-quality rLDPE, Green: good-quality rLDPE, light orange: quality control sample, and turquoise: blank samples.

By comparing good and poor-quality rLDPE extracts to vLDPE, 457 compounds were at least 10x more abundant in the poor-quality rLDPE extracts when compared to vLDPE. Additionally, 814 compounds have significantly different abundances between the good-quality and poor-quality rLDPE extracts.

Progenesis allows for a quick validation of the peak picking process for all annotated compounds. Figure 4 illustrates an example of *m/z* 441.2978 detected at 9.89 minutes. This ion is part of a compound cluster of [M+H]

$^{+}$ and $[M+Na]^{+}$. *m/z* 441.2978 corresponds to $[M+Na]^{+}$ (Figure 4).

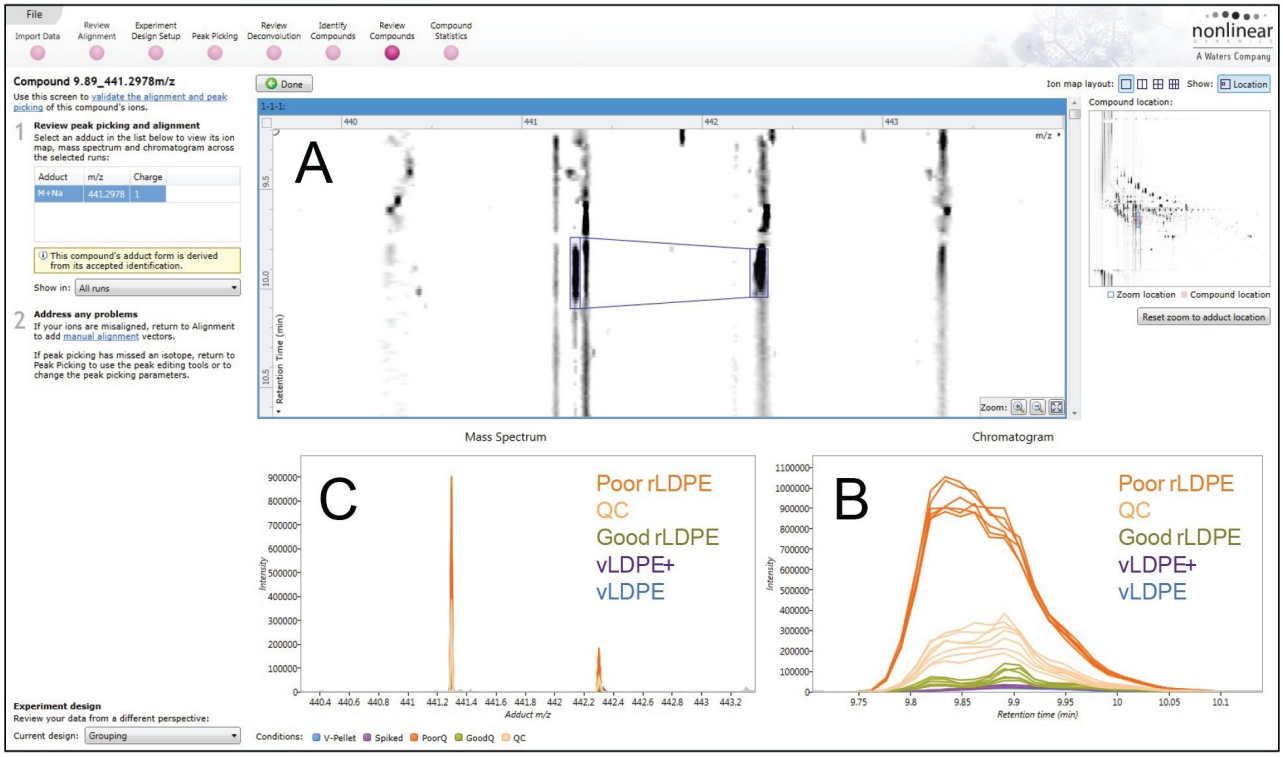

Figure 4. Peak picking by Progenesis QI. Figure 4A heat map of m/z vs retention time. The blue lines represent the ion cluster, containing the monoisotopic mass and first isotopic mass. Figure 4B Overlay of Extracted Ion chromatogram (EIC) of m/z 441.2978 in 24 injections. Figure 4C Overlay of the mass spectra in 24 injections. Each colour represents a sample group. Poor-quality rLDPE samples (dark orange traces) have the highest abundance, followed by the quality control samples (light orange traces), and the good-quality rLDPE extracts (green traces).

Figure 4 depicts the compound review from Progenesis QI. The top panel represents the heat map of *m/z* versus retention time (Figure 4A). The blue boxes surround the ion cluster of the compound of interest. The bottom right panel (Figure 4B) illustrates the Extracted Ion Chromatograms (EIC) for 24 injections of 4 sample groups. We can observe the reproducibility between the injections and the differences in abundance between the sample groups. Poor-quality rLDPE extract samples (dark orange traces) have the highest abundance, followed by the quality control samples (light orange traces) and the good-quality rLDPE extracts (green traces). Virgin pellets without

or with additives (blue and purple trances) have extremely low or no abundance of *m/z* 441.2978. The bottom left panel illustrates an overlay of the mass spectra in the 24 injections (Figure 4C). Herein, it can be concluded that the peak picking and retention time alignment of this molecular ion are correct.

In an attempt to identify these signature compounds, two relevant databases were used: Food Contact Chemicals (FCCdb, 3237 entries) and Chemicals associated with Plastic Packaging (CPPdb, 7149 entries) databases [Groh ***et al***. 2021 and Groh ***et al***. 2019, respectively].[4–5] These databases have 2353 compounds in common. Compound annotation was based on the accurate mass information, isotope similarity and fragments ion information when present. For compounds with no experimental fragmentation spectra in the database, Progenesis QI performed theoretical fragmentation based on the chemical structure and compared it to the experimental fragments measure by MS^E. An acceptable compound annotation is when the accurate mass of the parent ion is within ± 10 ppm and the fragments are within ±20 ppm. A confident compound identification was considered when the compound of interest had identical retention time and fragmentation spectra as the corresponding pure chemical standard [Schymanski ***et al***. 2015].[7]

In total 33 out of 457 compounds highly abundant in poor-quality rLDPE extracts, and 57 compounds out of 814 differentially expressed in good and poor-quality rLDPE extracts were annotated. Among the top hits were different phthalates such as: dinonyl phthalate/ diisononly phthalate, or octyl decyl phthalate, bis(2-propylheptyl)phthalate, didecyl phthalate /diisodecylphthalate, di-n-octyl phthalate, and a glyceryl monostearate.

Figure 5 shows an example for the annotation of *m/z* 441.2978 detected at 9.89 minutes based on accurate mass and fragmentation (generated by MS^E and compared to theoretical fragments).

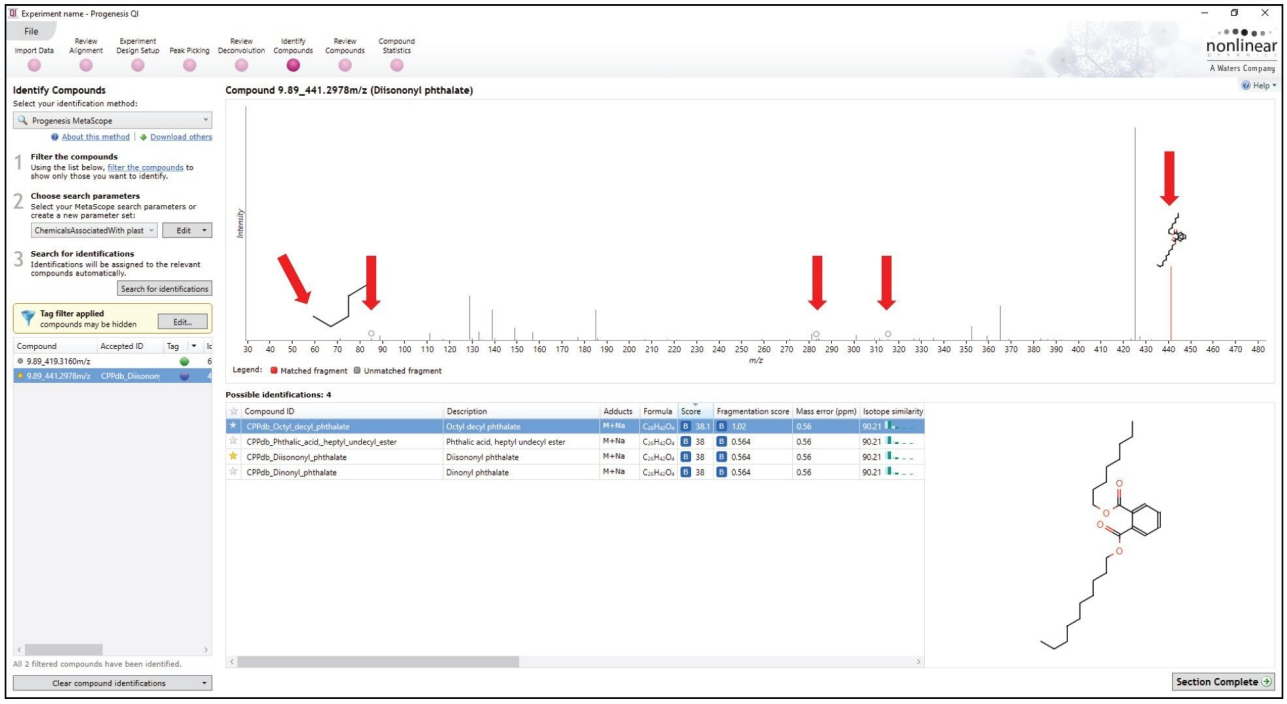

Figure 5. Annotation of m/z 441.2978 detected at 9.89 minutes. The list of potential compounds contains four isomers annotated using the CPPdb. The experimental fragments were annotated by comparison to theoretical fragmentation performed by Progenesis QI.

m/z 441.2978 corresponds to the sodium adduct of $C_{24}H_{38}O_4$ with mass accurcy ±0.56 ppm. This compound can potentially be any of the proposed four isomers. The fragments obtained by MS^E are annotated by comparison with ***in silico*** fragmentation patterns are highlighted by red arrows on Figure 5. These fragments are in common between the four suggested isomers of phthalates. Octyl-decyl-phthalate has the highest fragmentation score (Figure 5). Other high abundant fragments can be observed. Noteworthy, MS^E experiment generated fragments for other coeluting compounds. These highly abundant fragments are very likely not related to *m/z* 441.2978 and correspond to coeluting compounds among which the $[M+H]^+$ ions of this compound. The low abundance of the annotated fragments may be due to the difficulty in fragmenting a stable $[M+Na]^+$. Indeed, it is known that higher collisions energies are needed for fragmenting $[M+Na]^+$ molecular ions.

A level 1 identification is obtained by comparing the retention time and fragmentation patterns of the chemical standard and sample. [Schymanski ***et al*** 2015].[7] To reveal the real identity of *m/z* 441.2978 a pure chemical standard of Di-isononyl phthalate (DINP) and LDPE samples were analyzsed using the same chromatography

method followed by targeted MS/MS experiment.

The quadrupole isolation in a targeted MS/MS experiment of *m/z* 441.2978 permits eliminating other coeluting ions and fragmenting the isolated ions of interest (+/- 1 Da). This MS/MS experiments allowed for data comparison of the fragmentation pattern of *m/z* 441.2978 mass in both samples and pure standard injections.

DINP was detected as $[M+H]^+$ and $[M+Na]^+$ as *m/z* 419.3161 and *m/z* 441.2981 at 9.8 minutes. Figure 6 corresponds to the EIC *m/z* 441.298 in the different samples and in an injection of 1 µg/mL of DINP.

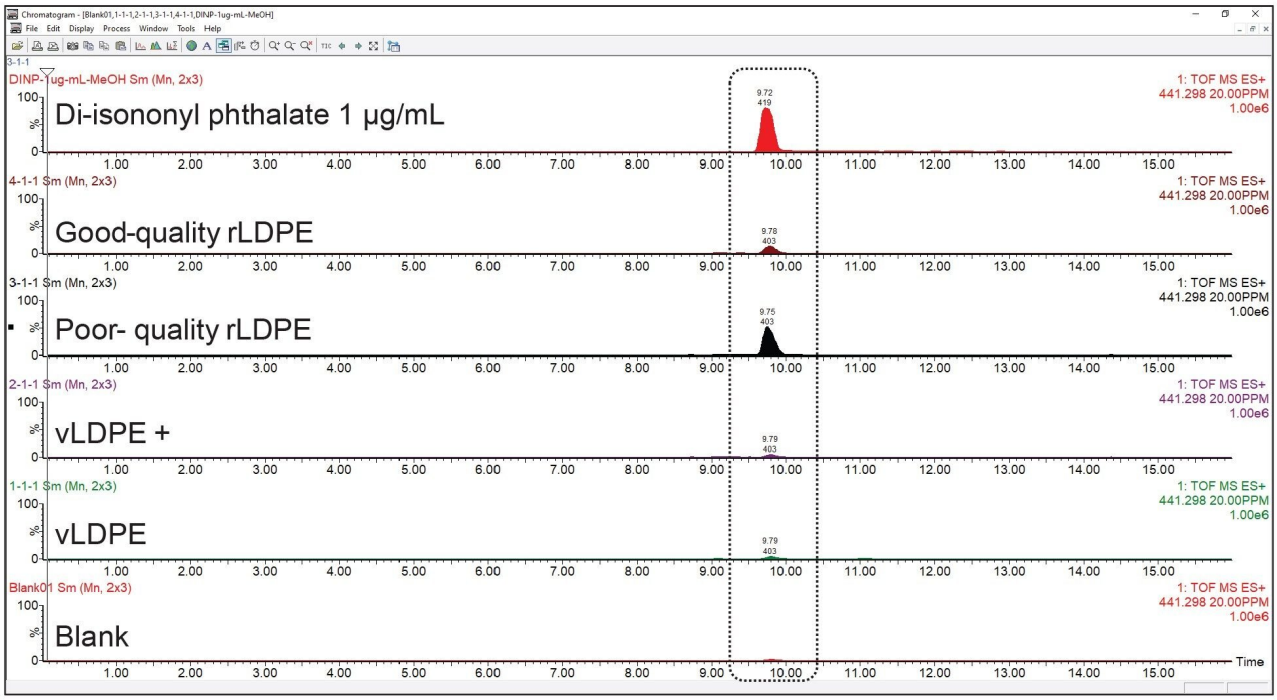

Figure 6. Extracted Ion chromatogram (EIC) of m/z 441.298 in samples and di-isononyl phthalate (DINP) standard. From bottom to top, EIC of a Blank sample, Virgin LDPE extracts (vLDPE), virgin LDPE extracts with additives (vLDPE+), poor-quality rLDPE, good-quality rLDPE, and a standard of 1 µg/mL of di-isononyl phthalate (DINP).

As indicated previously, *m/z* 441.298 is detected mainly in poor-quality rLDPE extracts, with small amount in the good-quality rLDPE extract and trace amounts in vLDPE extracts (with or without additives). The retention time of DINP is the same as *m/z* 441.298 in the different samples (Figure 6). To successfully fragment the sodium

adduct, higher collision energies were used (25–90 eV). Nevertheless, with collision energy as high as 90 eV *m/z 441.2956* is still the most abundant peak (Figure 7).

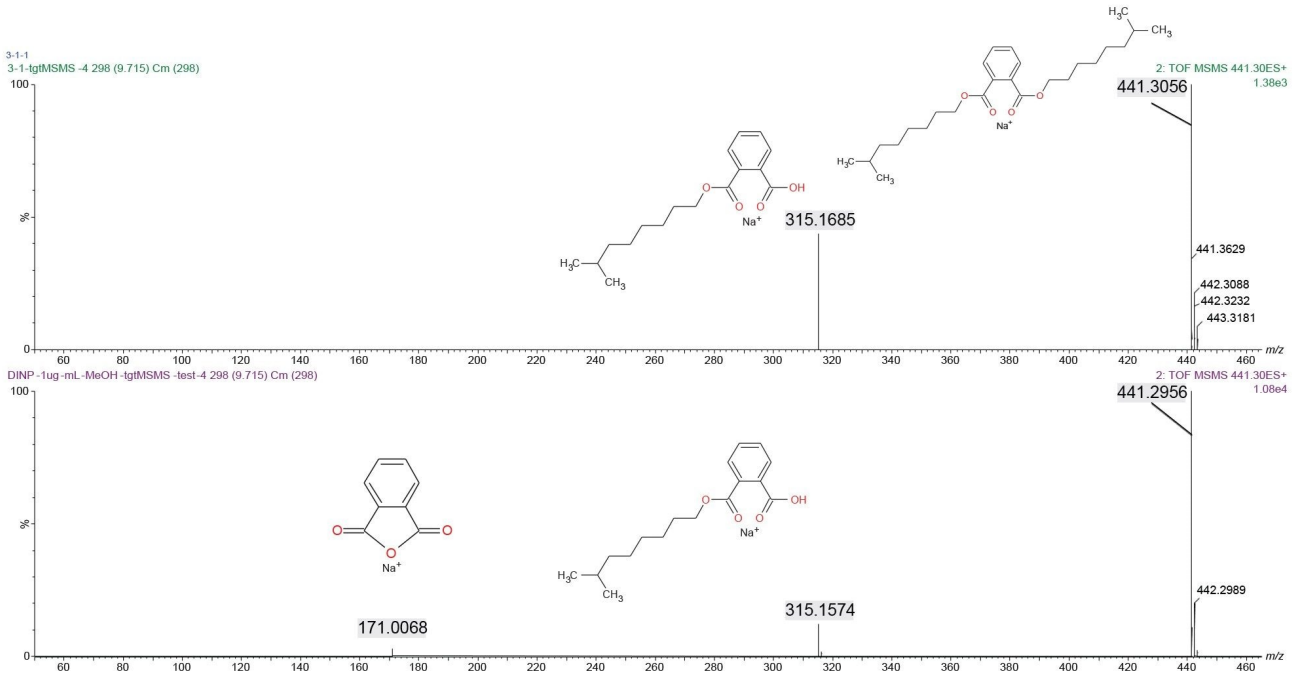

Figure 7. Comparison of fragmentation spectra of m/z 441.2956 in DNIP standard and in poor-quality rLDPE extracts using a ramped collision energy from 25–90 eV.

Fragmentation patterns for *m/z* 441 from both the standard and the poor-quality rLDPE extracts are very similar (Figure 7). In the poor-quality rLDPE extract *m/z* 171.0106 is not observed. This can be due to the actual concentration of the parent ion in the samples, compared to the amount in the standard (1 µg/mL). However, the signature fragment to phthalates *m/z* 149.0283 was observed in the $[M+H]^+$ fragmentation spectra (data not shown). As expected, DINP sodium adduct is very stable. It required collision energy up to 90 eV to break the stable structure. Therefore, once fragments are generated, they are detected as sodium adducts. The structures on figure 7 are suggestions for the fragment attribution.

Herein, by using a chemical standard, and comparing the retention time and fragmentation patterns of both the protonated and sodiated adducts, the compound detected *m/z* 419.3161 and *m/z* 441.2981 is confidently identified as DINP. By applying the same approach, several additional compounds were identified: diisononyl phthalate

(DINP), di-isobutyl phthalate (DIBP), di-n—octyl phthalate (DNOP), and oleamide.

Conclusion

In this application note, a novel analytical approach was employed using ACQUITY Premier LC coupled to Xevo G3 QTof high resolution mass spectrometry and software tools to distinguish qualities of rLDPE.
By analysing the migration compounds of different batches at least four significant marker compounds were identified in positive ESI mode that can be used to distinguish poor-quality rLDPE from good quality rLDPE. In addition, this method can be easily transferred for absolute quantitation of these compounds using tandem mass spectrometry.

References

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