

Analysis of Polylactic acid Filters

Response to the article entitled “iQOS:
evidence of pyrolysis and release of a toxicant
from plastic” by *Davis B. et al., 2018*

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Philip Morris International R&D

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1 EXECUTIVE SUMMARY

Recently, Davis B. *et al.* (University of California, Department of Molecular, Cell and Systems Biology) have reported the analysis of polylactic acid (PLA) filters of iQOS heatsticks by means of headspace with subsequent gas chromatography-mass spectrometry (nominal mass resolution) analysis [1]. Amongst several findings, they have reported the release of formaldehyde cyanohydrin (CAS # 107-16-4), an acute toxicant, according to:

- Mass spectral matching (acceptance criteria >85%) towards the spectral library of National Institute of Standard and Technology (NIST)
- No further confirmation step was performed (e.g. by injection of the purchased analytical grade reference standard) in order to unambiguously prove its presence.

To verify the hypothesis stated by the authors, Philip Morris International, R&D repeated the analytical setup using headspace analysis coupled to gas chromatography high resolution Time-of-Flight mass spectrometry (HS-GC-HR-MS).

While both approaches showed good congruence of the obtained chromatographic fingerprints (relative abundancies and elution order), discrepancies were observed regarding the compounds identification. Specifically, Philip Morris International, R&D, could demonstrate:

- Difference of the chromatographic retention time (linear retention index) of formaldehyde cyanohydrin obtained from the injection of the analytical grade reference standard and the suspected compound observed in the PLA filter headspace analysis
- No trace of formaldehyde cyanohydrin in the overall PLA filter chromatogram while searching for fragment ions obtained from the reference standard
- Confirmed characterization of the chemical identity of the suspected peak as meso-lactide (CAS # 13076-19-2), a known condensation product of lactic acid.

Thus, Philip Morris International, R&D could unambiguously demonstrate the absence of formaldehyde cyanohydrin in the headspace analysis of PLA filter.

2 INTRODUCTION

The Department of Molecular, Cell and Systems Biology, University of California, Riverside, USA has recently published an article in Tobacco Control [1] detailing, amongst other aspects, the analysis of polylactic acid (PLA) filters of iQOS heatsticks using headspace (HS) coupled with gas chromatography-mass spectrometry (GC-MS) to assess if potentially toxic chemicals were emitted

from the filter during heating. From their analysis, the authors highlighted the presence of cyanohydrin formaldehyde (glycolonitrile), an acute toxicant.

This report aims to summarize the scientific approach led by Philip Morris International (PMI) R&D to verify the hypothesis stated by the University of California. Thus, the analytical steps conducted within PMI R&D for the analysis of PLA filters from iQOS heatsticks using headspace analysis coupled to high resolution Time-of-Flight mass spectrometry (HS-GC-HR-MS) and the subsequent identification of compounds determined in this HS portion will be described and the outcomes will be shared unambiguously.

It has to be pointed out that the term ‘compounds identified’ or ‘identified compounds’ is defined differently between PMI R&D and University of California. Indeed, University of California used the term ‘compounds identified’ or ‘identified compounds’ when achieving the threshold criteria (> 85 %) set for mass spectral matching towards the spectral library of National Institute of Standard and Technology (NIST). Within PMI R&D, compounds identities are first postulated from both accurate mass spectra together with predicted linear retention indices ($LRI_{\text{predicted}}$) before being confirmed by results obtained from injection of purchased analytical grade reference standards [2], a fundamental step that the University of California has not performed.

3 COMPARISON OF ANALYTICAL METHODOLOGIES

3.1 HS-GC-MS APPROACH (UNIVERSITY OF CALIFORNIA)

For this study, investigators at the University of California purchased Marlboro (blue box) heatsticks, manufactured by Philip Morris Brands Sàrl (Italy). The methodology employed for analysis of the iQOS heatstick PLA filter comprises a headspace sampling of the filter followed by GC-MS analysis using nominal mass quadrupole analyzer. For headspace analysis, the PLA filter was removed from unused heatsticks and a 3 mm portion closest to the tobacco plug was excised and placed in a 20 mL headspace vial; no further information on the headspace sampling parameters are detailed in the paper [1]. The chromatographic separation was accomplished using an Agilent J&W HP-5ms Ultra Inert GC Column (30 m x 0.25 mm x 0.25 μm) and subsequent detection of the compounds using an Agilent 5977A MSD, employing electron ionization (EI) at 70 eV in positive acquisition mode. The identification of compounds was performed by comparison of the obtained mass spectra with the mass spectral library of the National Institute of Standard and Technology (Gaithersburg, Maryland, USA) while listing the best hit as identified compound when showing a 85% or higher probability match factor provided by the acquisition software.

3.2 HS-GC-HR-MS APPROACH (PHILIP MORRIS INTERNATIONAL, R&D)

PMI R&D analyses were realized using HS-GC-HR-MS. As described by the University of California, the PLA filter was removed from three unused heatsticks and a 3 mm portion closest to the tobacco plug was excised and placed in a 20 mL headspace vial with subsequent heating phase at 100°C for 10 min prior to injection. A series of odd n-alkanes (used as reference index markers) was added to two PLA sample replicates prior headspace sampling to allow for linear retention indices (LRI) determination. Chromatographic separation was performed on an Agilent J&W DB-624 Ultra Inert GC Column (30 m x 0.25 mm x 1.4 μ m) coupled to an Agilent 7200A Q-TOF high resolution mass spectrometer, according to the conditions already described by Dossin E. *et al.* [2]. The identification of the compounds was conducted by determination of the elemental composition of fragment ions, comparison of background subtracted EI mass spectra to the mass spectral libraries of the National Institute of Standard and Technology (version 2014) and Wiley (version 2011), by evaluation of calculated LRI versus predicted values of postulated compounds resulting from MS library search, and finally confirmation by means of purchasing and analysis of analytical grade reference standards [2].

4 RESULTS

4.1 HS-GC-MS APPROACH (UNIVERSITY OF CALIFORNIA)

As can be seen from Figure 1, the University of California stated the identification of four compounds present in the headspace portion of PLA filter, namely formaldehyde cyanohydrin (RT 17.97 min), ϵ -caprolactone (RT 18.05 min), lactide (RT 19.63 min) and 1,2-diacetin (RT 24.98 min).

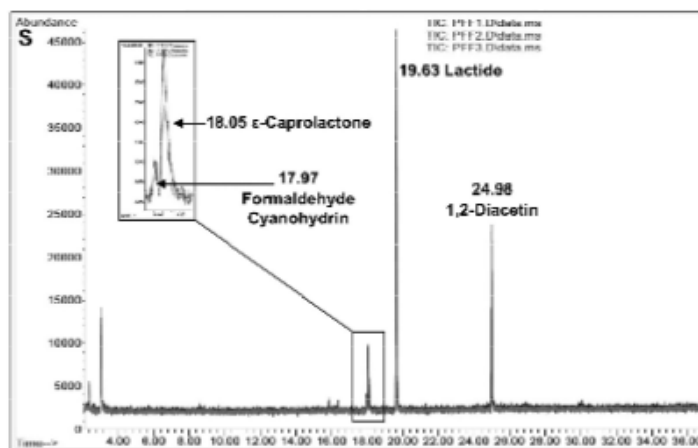


Figure 1: University of California, GC-MS spectrometry headspace analysis of unused PLA filter. Total ion chromatogram (TIC) shows an overlay of three runs, relative abundance was plotted versus retention time in minutes. Inset shows a magnified view of peaks with close retention times [1].

4.2 HS-GC-HR-MS APPROACH (PHILIP MORRIS INTERNATIONAL, R&D)

As can be seen from Figure 2, PMI R&D analysis revealed, in congruence to the results obtained by the University of California, the presence of four peaks in the headspace portion of PLA filter, eluting at retention times of 16.38, 16.47, 17.14, and 18.58 min, respectively (PMI conditions). LRI values for the four peaks were respectively calculated with 1265, 1271, 1316, and 1422. The chromatographic pattern is closely related to the one obtained by the University of California (Figure 1). The compounds eluting at retention times of 16.38, 16.47, 17.14, and 18.58 min were univocally confirmed as meso-lactide (LRI 1265, CAS # 13076-19-2), ϵ -caprolactone (LRI 1271, CAS # 502-44-3), lactide (LRI 1316, CAS # 95-96-5) and triacetin (LRI 1422, CAS # 102-76-1) by means of comparison with analysis obtained from purchased analytical grade reference standards.

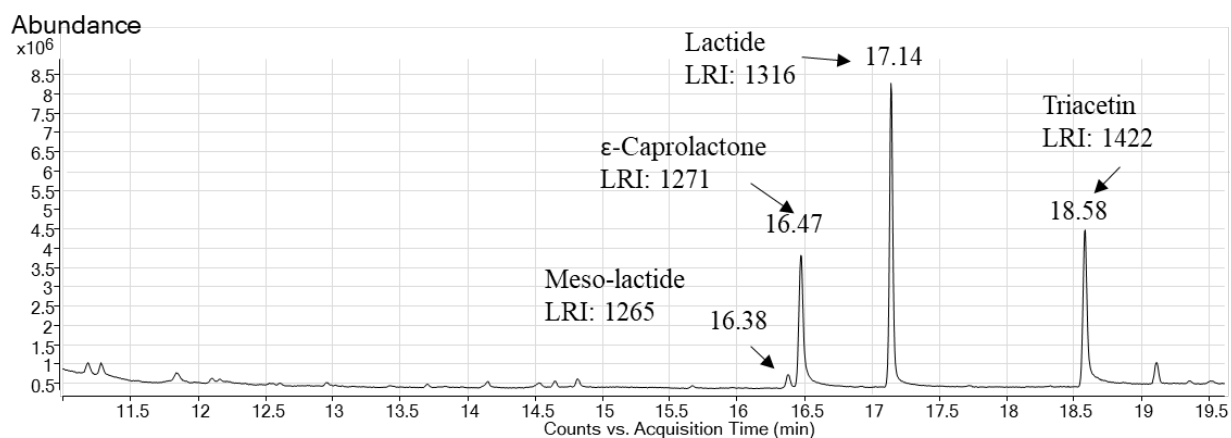


Figure 2: Philip Morris International, R&D, GC-HR-MS headspace analysis of unused PLA filter. Total ion chromatogram (TIC) shows an overlay of two runs, relative abundance was plotted versus retention time in minutes (identity of confirmed compounds have been ascribed as well as their calculated LRI values).

The analytical grade reference standard formaldehyde cyanohydrin (CAS # 107-16-4) was purchased from Sigma (named as glycolic acid nitrile solution, article number 50640) and GC-HR-MS analysis was performed using the same analytical conditions, besides liquid injection. The obtained total ion chromatogram (TIC) as well as the related mass spectrum are displayed in Figure 3 and revealed a retention time for formaldehyde cyanohydrin of 11.53 min, being clearly different from the suspected peak observed at 16.38 min from the PLA analysis (Figure 2).

The subsequent screening with the experimentally determined formaldehyde cyanohydrin specific ions in the overall PLA headspace chromatogram did not reveal any trace of these ions and thus confirmed absence of formaldehyde cyanohydrin.

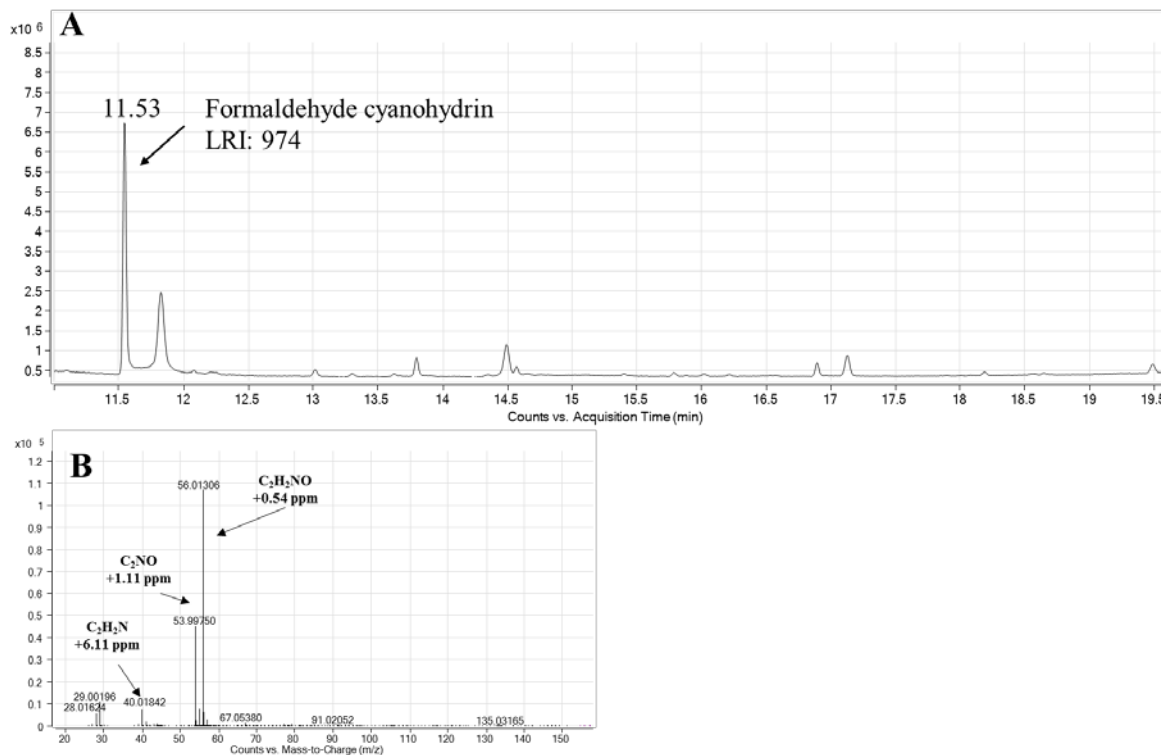


Figure 3: Philip Morris International, R&D, A) GC-HR-MS analysis of the analytical grade reference standard formaldehyde cyanohydrin (CAS # 107-16-4) spiked with a series of odd *n*-alkanes for LRI determination; B) background subtracted EI accurate mass spectrum of formaldehyde cyanohydrin (CAS # 107-16-4) eluting at RT 11.53 min (calculated LRI value of 974). The proposed elemental composition of the major fragment ions are reported together with their mass precisions.

5 DISCUSSION

From the methodology and data presented in the publication by University of California, it is obvious that, when compared with PMI's approach regarding the characterization of PLA filter using headspace, erroneous identifications were proposed by the University of California for two compounds (namely formaldehyde cyanohydrin and 1,2-diacetin eluting at 17.97 and 24.98 min, respectively).

While both approaches showed good congruence of the chromatographic fingerprints (relative abundancies and elution order) of the PLA filter analyzed by headspace injection mode, discrepancies were observed regarding their compounds identification. The presence of formaldehyde cyanohydrin in the peak eluting at a retention time of 17.97 min (University of California) and of 16.38 min (PMI R&D) was excluded from the data generated by PMI R&D both from the non-matching retention times (reference standard formaldehyde cyanohydrin eluted at 11.53 min with calculated LRI value of 974) and from the mass precision of the ions observed from the peak eluting at 16.38 min. Furthermore, it

is worth noticing that no trace of this compound was observed in the overall analysis of PLA filter samples when characteristic formulae search of C_2H_2N , C_2NO , and C_2H_2NO related to formaldehyde cyanohydrin (Figure 3b) were extracted within ± 20 ppm tolerance mass window.

This finding is in line with PMIs expectations as this compound was as well not detected during routine material evaluation screening by means of volatile organic compound (VOC) determination upon heating followed by GC-MS analysis.

Figure 4A depicts the GC-HR-MS headspace analysis of PLA filter sample with special emphasis on the peaks observed at retention times of 16.38 and 17.14 min (LRI of 1265 and 1316, respectively). Their corresponding EI accurate mass spectrum (background subtracted) revealed strong similarities matching both with the presence of C_2H_3O and C_3H_4O moieties (Figure 5B & C). MS library search provided (D,L)-lactide as the best compound hit for RT 16.38 min using Wiley (version 2011) with a matching factor of 89.9%, although the analytical grade reference standard compound (CAS # 95-96-5) eluted under our condition at a RT 17.14 min (LRI 1316).

Knowing that lactide compound is obtained from the cyclisation of two lactic acid monomers, we investigated the analysis of a relatively high concentration of lactic acid reference standard (CAS 50-21-5, $C_3H_6O_3$). Figure 4D depicts the GC-HR-MS analysis of lactic acid standard with the extracted ion chromatogram of m/z 56.0260 (corresponding to C_3H_4O) using the same retention time range as for the PLA filter.

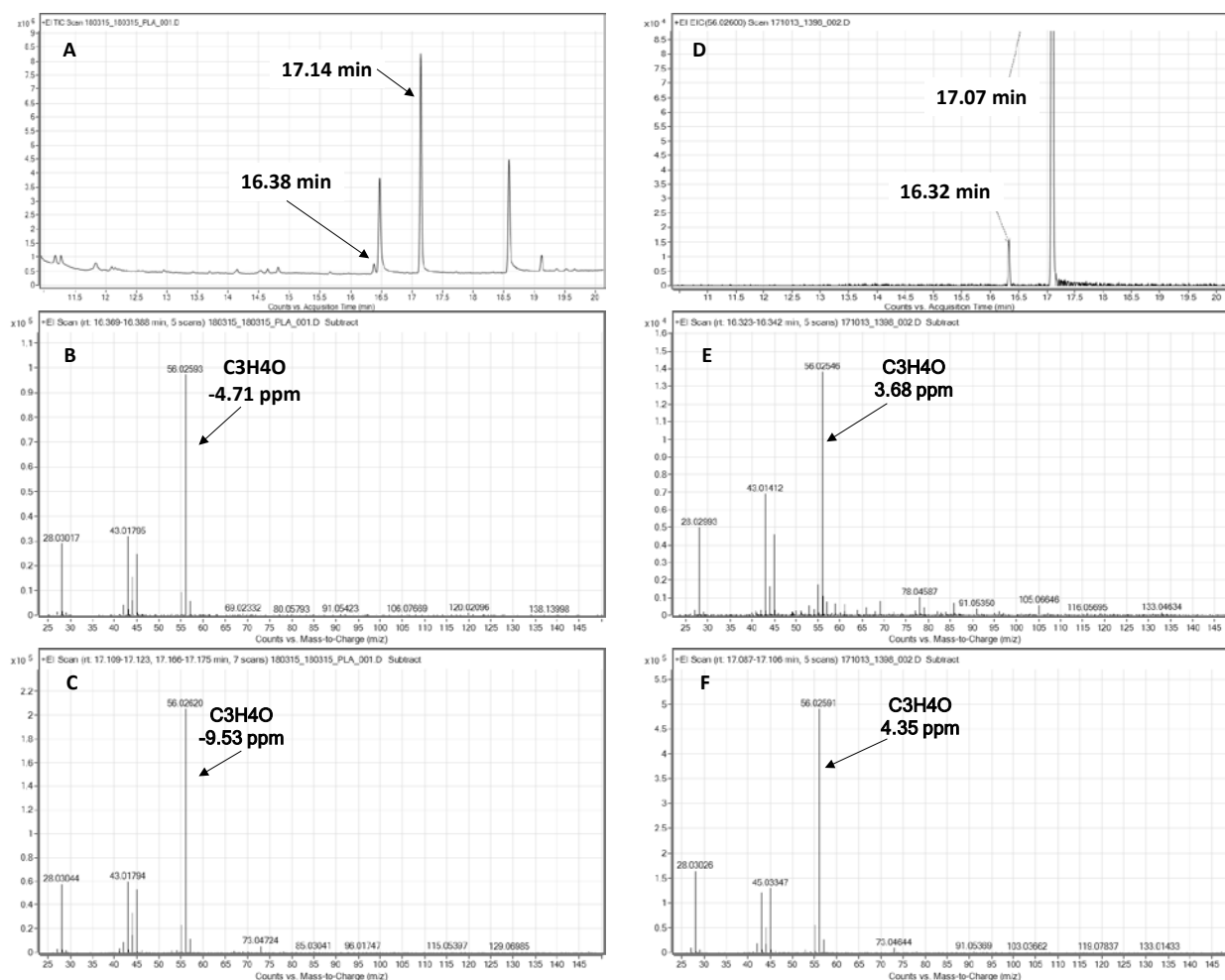


Figure 4. Philip Morris International, R&D, A) GC-HR-MS headspace analysis of unused PLA filter with emphasis on peaks eluting at RT 16.38 and 17.14 min. B & C) Background subtracted EI accurate mass spectra related to RT 16.38 and RT 17.14 min, respectively. D) Extracted ion chromatogram of m/z 56.0260 (corresponding to C₃H₄O, using a mass extraction window of 20 ppm) from the analysis of concentrated solution of lactic acid analytical grade reference standard solution (CAS # 50-21-5) with emphasis of peaks eluting at RT 16.32 and 17.07 min. E & F) Background subtracted EI accurate mass spectra related to RT 16.32 and RT 17.07 min, respectively.

In this case, our interest was not to show the peak corresponding to lactic acid (RT 8.0 min, LRI 794.56), but to pay attention on the presence of dimer associated to lactide species. Obviously, a major peak is noticed in this chromatographic region at RT 17.07 min, whereas a second minor peak eluted at RT 16.32 min (Figure 4D)¹. Figure 4E & F depict background subtracted EI accurate mass spectra of both peaks, suggesting a strong similarities with those observed from the PLA filter (corresponding RT values shown in Figure 4B & C).

¹ The lactic acid standard compound was analyzed in October 13th 2017, therefore a slight shift in retention times is observed compared to PLA filter analysis (but showed similar LRI values).

These results strongly support the evidence of the presence of lactide isomers, which has been already reported by Arrieta MP *et al.* [3]. Indeed, the cyclisation product resulting from two lactic acid monomers generated two peaks chromatographically separated by gas chromatographic conditions with 1.2 min retention time difference (meso-lactide: RT 16.2 min and lactide: RT 17.4 min); under their temperature gradient conditions using a HP-5ms GC column (30 m x 0.25 mm, 0.25 μ m).

As lactide is known to be present as three isomers, namely (D)-lactide, (L)-lactide and meso-lactide the respective reference standards were purchased and analyzed. While the former two compounds co-eluted under our analytical conditions (RT 17.14 min), meso-lactide eluted earlier at RT 16.38 min. By means of spiking experiments in addition to the data obtained from the accurate mass spectra (Figure 5 A-F), the compound eluting at RT 16.38 min in the PLA headspace chromatogram was univocally identified as meso-lactide.

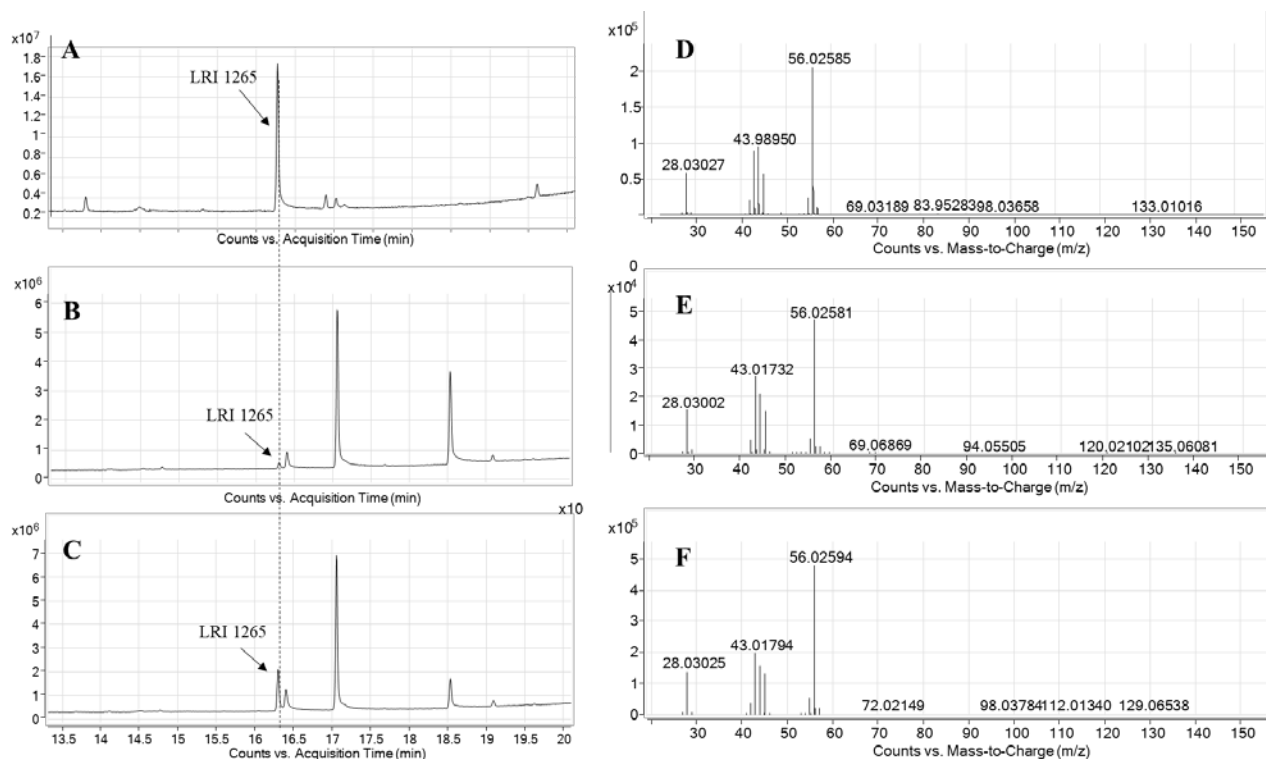


Figure 5. Philip Morris International, R&D, A) GC-HR-MS analysis of meso-lactide reference standard solution (NatureWorks, CAS # 13076-19-2) B) GC-HR-MS headspace analysis of unused PLA filter C) GC-HR-MS headspace analysis of unused PLA filter spiked with meso-lactide reference standard solution D) Background subtracted EI accurate mass spectra related to LRI 1265 of meso-lactide reference standard solution, E) Background subtracted EI accurate mass spectra related to LRI 1265 of headspace analysis of unused PLA filter, F) Background subtracted EI accurate mass spectra related to LRI 1265 of headspace analysis of unused PLA filter spiked with meso-lactide reference standard solution

The second discrepancy refers to 1,2-diacetin (reported by University of California) as opposed to triacetin (confirmed by PMI R&D). EI mass spectra of both compounds resulted in similar fragment ions as provided by NIST 14. Indeed, the difference between these two compounds is based on the addition of an acetate moiety for triacetin (C₉H₁₄O₆, CAS #102-76-1) as compared to 1,2-diacetin (C₇H₁₂O₅, CAS # 102-62-5). In such case, accurate mass measurement cannot distinguish between the two compounds and only chromatographic data of both analytical grade reference chemicals is able to unambiguously confirm the proper compound identity.

These result discrepancies highlight the importance to incorporate chromatographic evaluation scoring when postulating compound identification, especially if no analytical grade reference standard is analyzed using same conditions. Even if the NIST and/or Wiley library matching score is close to 100%, wrong compound proposal can still occur, as already reported by Schymanski E.L. *et al.* [4].

However, investigation conducted at the University of California and internally at PMI concluded on the same chemical identity for the compounds ϵ -caprolactone and lactide, eluting at retention times of 18.05 and 19.63 min (University of California, Figure 1) and at 16.47 and 17.14 min (PMI R&D, Figure 2).

6 CONCLUSION

The identification of compounds released from PLA filters as reported by the University of California is partly erroneous. No release of formaldehyde cyanohydrin from PLA filters of iQOS heatsticks is observed, but the suspected peak was univocally identified as meso-lactide (CAS # 13076-19-2) by analysis of the reference standard compound as well as in spiking experiments. Furthermore, triacetin was determined to be present in the headspace of PLA instead of 1,2-diacetin.

The overall accuracy for compound identification as performed in this study by Davis B. *et al.* (University of California) was proven to be insufficient and lacking for additional supplementary analysis (injection of reference standards) as well as information (e.g. absence of EI mass spectra for the four peaks highlighted in their publication). Obviously, a wrong analytical data interpretation leads to a wrong conclusion with misleading message as highlighted by the authors.

7 REFERENCES AND RELATED DOCUMENTS

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5. PMI Unpublished on file data. PMI-RRP-WKI-111615 – Untargeted screening of volatile and semi-volatile compounds using GC-HR-MS

8 ABBREVIATIONS

CAS	:	Chemical Abstracts Service
EDMS	:	Electronic Data Management System
EI	:	Electron Ionization
GC-MS	:	Gas Chromatography-Mass Spectrometry
HS-GC-HR-MS	:	HeadSpace-Gas Chromatography-High Resolution-Mass Spectrometry
HS-GC-MS	:	HeadSpace-Gas Chromatography-Mass Spectrometry
iQOS	:	I Quit Original Smoking
LRI	:	Linear Retention Index
min	:	Minute
mL	:	Milliter
NIST	:	National Institute of Standards and Technology
PLA	:	Atmospheric Pressure Chemical Ionization
PMI, R&D	:	Philip Morris International, Research and Development
ppm	:	Part Per Million
Q-TOF	:	Quadrupole-Time Of Flight
RT	:	Retention Time
TIC	:	Total Ion Chromatogram
TOF	:	Time Of Flight
VOC	:	Volatile Organic Compounds
WKI	:	Work Instruction